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ERRATA

VOLUME I

Page 163, line 6 from foot of page, "CO_3" should read "SO_3".

Page 392, Table VI, heading of first column, "Incr. 1 c.c. over 2.0 c.c." should read "Incr. 1 c.c. over 0.2 c.c."; heading of second column "Incr. 1 c.c. over 1.0 c.c." should read "Incr. 5 c.c. over 1 c.c."; heading of third column "Incr. .5 c.c. over 0.2 c.c." should read "Incr. 1 c.c. over 0.2 c.c."

Page 505-508. "A Rapid Method for the Estimation of Calcium Oxide in Peat Soils," by R. A. Gortner. For index references, see Index of Vol. II.

Page 541, line 11 from bottom, "Oudenmans" should read "Oudemans."

Page 571, lines 6 and 7. "The effect of reaction on ammonification by these fungi is more pronounced in clay than in sandy soil" should read "The effect of reaction on ammonification by these fungi is more pronounced in sandy than in clay soil."

VOLUME II

Page 2, line 15, "Oudmann" should read "Oudemans."

Page 63, reference 9, "Keimghabt" should read "Keimghalt."

Page 64, reference 13, "Denitrifikateonsbakterien" should read "Denitrifikationsbakterien."

Page 102, legend for Plate I, figure 2, "or" should read "on."

Page following page 102, legend for Plate II, figure 2, "bed on right" should read "bed on left," and "bed on left" should read "bed on right."

Page 112, line 2, "48 per cent of iron" should read "48 per cent of Fe_2O_3".

Page 112, line 22, "indentification" should read "identification."

Page 118, line 2 in table, *"Peiicillium atramentasum"* should read *"Penicillium atramentosum."*

Page 118, line 17 from foot of table, *"Dematuim pallulans"* should read *"Dematium pullulans."*

Page 129, line 15 from foot of page, "Tukal" should read "Zukal."

Page 131, line 10, "medicum" should read "medium."

Page 135, line 13 from foot of page, *"cinnabarimus"* should read *"cinnabarinus."*

Page 137, line 22, *"pollulans"* should read *"pullulans."*

Page 144, line 3 from foot of table, *"roseum"* should read *"roseum."*

Page 144, last line in table, *"pollulans"* should read *"pullulans."*

Page following page 156, legend for Plate IV, fig. 3, "canidiophores" should read "conidiophores."

Page following page 156, legend for Plate V, fig. 11, "Fuckii" should read "Fuckelii."

Page 167, line 12, "hard" should read "hand."

Page 217, line 3, "3.0" should read "3.5."

Page 217, figure 1, "atmosphe es" should read "atmospheres."

Page 221, line 24, "1 tenth" should read "2 tenths."

Page 221, line 25, "2 tenths" should read "1 tenth."

Page 225, figure 2, "calci m" should read "calcium."

Page 230, figure 4, lettering across foot of cut should read: upper line, "R, R 2, R 3, R 4, R 5, R 6, R 7, R 8," and lower line "C 1, C 2, C 3, C 4, C 5, C 6, C 7, C 8, C 1, C 2, C 3, C 4, C 5, C 6, C 7, C 1, C 2, C 3, C 4, C 5, C 6, C 1, C 2, C 3, C 4, C 5, C 1, C 2, C 3, C 4, C 1, C 2, C 3, C 1, C 2, C 1.

Page 245, footnote, next to last line, "MgO" should read "Mg."

Page 248, line 28, insert "ratio" after "calcium-magnesium"

Page 252, reference 28, "Oesterhout" should read "Osterhout."

Page 258, line 17, *"Bacillus Rutida"* should read *"Bacillus Putida."*

Page 265, add footnote: "Per cent acidities are represented in terms of normal, rather than concentrated acid."

Page 266, line 11, "Stömer" should read "Störmer."

Page 271, figure 3, legend, add "(Series 2)."

Page 338, figure 6, legend, "CaO_3" should read "$CaCO_3$".

Page 411, Table VII, third entry, "Carbonate CO" should read "Carbonate CO_3".

Page 437, footnote, line 19, "sols" should read "soils."

Page 481, line 25, equation (2) "a" should read *"α"*.

Page 485, line 2, "$\log \frac{5.56}{184.27}$" should read "$\log \frac{5.55}{184.28}$".

Page 488, line 9, two lines following Table III should immediately precede the table.

Page 496, line 3, "phosporus" should read "phosphorus."

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VOL. II JULY, 1916 NO. 1

SOIL SCIENCE

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SOIL SCIENCE

A · MONTHLY · JOURNAL · DEVOTED TO · PROBLEMS · IN · SOIL · PHYSICS SOIL · CHEMISTRY · AND · SOIL · BIOLOGY

Volume II Number 1

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SOIL SCIENCE

RUTGERS COLLEGE

VOL. II NEW BRUNSWICK, N. J., JULY, 1916 No. 1

ENVIRONMENTAL FACTORS INFLUENCING THE ACTIVITY OF SOIL FUNGI[1]

BY

DAVID AUGUSTUS COLEMAN, *Research Fellow, Rutgers College*

INTRODUCTION

The study of soil biology in its relationship to soil fertility has been the subject of research by numerous investigators. Included in this branch of soil science as stimuli for investigation are the many microscopic inhabitants of the soil, viz., algae, bacteria, fungi and protozoa. Up to within comparatively recent times the soil biologist has concerned himself mainly with the study of soil bacteria, giving only casual attention to the other microflora and fauna of the soil.

The work of Russell and Hutchinson (35, 36) of the Rothamsted Experiment Station has given rise to extensive experimentation dealing with the influence of soil protozoa on soil fertility. In time, therefore, we shall have a very comprehensive knowledge of the possible rôle that these organisms might have on the crop-producing power of the soil.

The other microörganisms in this field have not been the subject of so intimate an investigation. Early researches by Müntz and Coudon (30) and likewise Marchal (27) have shown that fungi have the power to decompose organic matter with the production of ammonia. More recently there appeared a paper by McLean and Wilson corroborating the above and also giving additional data as to the effect of the chemical and physical properties of the soil on the activity of soil fungi. A few investigators have also endeavored to demonstrate the azofying (21) and azotification powers of these organisms. The balance of evidence seems to be on the negative side. The early as well as recent investigations on the ammonifying efficiency of soil fungi indicates that they might play an important rôle in controlling soil fertility. It becomes of interest therefore, to gain some insight into the forces which control the life processes of these organisms.

[1] A thesis presented to the faculty of Rutgers College in partial fulfillment of the requirements for the degree of Master of Science in Research, 1916.

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The subject chosen for research expresses quite clearly the objective to be obtained. Higher plants are dependent in a large measure upon soil microörganisms for their food. Studies then, of the conditions which affect the activity of these organisms are highly desirable.

Historical Resumé

The history of the general subject of the factors that might influence the activity of soil fungi is not of an extended nature. A survey of the literature seems to indicate that the reaction of the medium has much to do with the activity of these organisms. Marchal (27) relates that soils having a weakly alkaline or neutral reaction have relatively few fungi. Ramann (34) and his co-workers have shown, that in a forest soil with which they experimented the proportion of fungi to bacteria was 3 to 1! These investigators also found high numbers of fungi in soils especially rich in humus and of a high acid reaction. Fischer (8), Faelli (9), Oudmann and Köning (31) and others have also recorded the presence of fungi in rich acid soils.

The relationship of acidity to the numbers and activity of soil fungi is quite favorably brought out in a paper by Hall, et al (12), of the Rothamsted Experiment Station. These authors give data showing the relatively large numbers of fungi in the plots that had become acid on account of continuous application of ammonium sulphate. The presence of organic matter in the soil also seems to be a factor influencing the activity of soil fungi. Marchal (30) gives data recording the presence of relatively few fungi in soils that had been extensively cultivated, or soil containing a small proportion of organic matter. Fischer (8), on the other hand, found that the addition of manure resulted in an excellent medium for the activity of soil fungi. More recently, McLean and Wilson (26) give figures to show the influence of the quality of the organic matter on the activity of soil fungi. The same authors further indicate that the chemical composition of the soil also controls the activity of these organisms. This was especially true when soluble phosphates where present. Numerous other investigators among whom are Löhnis (25), Gerlach and Vogel (11), Jensen (13), Butkewitsch (2), have shown the favorable influence of ammonium salts and of nitrate upon soil fungi.

A recent paper of Waksman and Cook (39), in which the authors endeavor to study the moisture factor in relation to a representative of each of three groups of prominent soil fungi, leads to the conclusion that the optimum moisture content for soil fungi activities is near the optimum for the soil.

It is of interest to note in passing that Kappen (14) and lately Löhnis (25), have shown the possibility of fungi aiding the transformation of cyanamid nitrogen into ammonia. It is evident, then, that much more data needs to be tabulated before any definite conclusions can be drawn regarding the activities of these organisms.

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Methods Used

The soil culture method as recommended by Lipman and Brown (22) for the measurement of the activities of soil microörganisms, modified by the sterilization of the soil was employed throughout this work. Ammonia production and its subsequent accumulation has been shown to be a measure, within certain limits of biological activities. In discussing results in this paper the production of ammonia will be used synonomously with activity, i. e., increased activity meaning increased accumulation of ammonia.

One-hundred-gram portions of soil were weighed out, quantities of organic matter equivalent to 155 mg. of nitrogen were added to each portion and the whole thoroughly shaken in a hand shaker identical with those used for the preparation of egg drinks at soda fountains. This device is fully described by us in another place (18). The contents of the shaker were transfered to a 200-c.c. Erlenmeyer flask and the proper moisture supplied, taking cognizance of the amount of moisture lost in the sterilization process. The flasks were then plugged with cotton and sterilized in the autoclave at 15 pounds pressure for 15 minutes. It has been shown by Coleman, Lint and Kopeloff (3) that this was the only means at present that we have for totally eliminating other biological factors. Upon cooling, the flasks were inoculated with 1 c.c. of a liquid culture of fungus spores.

After incubating 7 days at 22° C. the soil was transfered to copper flasks with 250 c.c. of tap water, a piece of paraffin and an excess of heavy magnesium oxide added, and the ammonia distilled over into N/10 HCl and titered with N/10 NaOH, using cochineal as the indicator.

The fungus spores were obtained by inoculating 100 c.c of Cook and Taubenhaus' (6) medium No. 2 with some of the fungus to be studied and incubating at 22° C. until an abundance of spores had been formed. This usually took from 7 to 12 days, species of *Penicillium* and *Trichoderma* requiring longer periods for sporulation.

The composition of Cook's (6) medium is as follows:

Distilled water	1000 c.c.
Glucose	20.00 gm.
Peptone	10.00 gm.
K_2HPO_4	.25 gm.
$MgSO_4$	.25 gm.

At the end of the incubation period the flask containing the fungus was shaken vigorously for 10 minutes to dislodge as large a number of spores as possible. The liquid was then transfered to a second sterile flask containing sand and again shaken to break up clumps of spores, the number of spores was then counted by the method devised by Kopeloff,

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Lint and Coleman (17) for the enumeration of soil protozoa. As the fungus spores were not motile the magnitude of the error would be considerably less than noted in the last mentioned article.

As a check upon the purity of the cultures, as well as on the efficiency of sterilization, a sterile tube of bouillon was inoculated with a small portion of the soil at the end of the incubation period. At the end of 24 hours these tubes were examined for bacterial contamination by plating upon Lipman and Brown's (23) synthetic agar.

The Soils Used

The soils used in these investigations were of two types, one a sandy loam designated by the Bureau of Soils of the United States Department of Agriculture as a Norfolk sandy loam and the other a clay loam, known as a Penn clay loam. The Norfolk sandy loam had a lime requirement according to the Veitch method for soil acidity of 400 pounds of calcium oxide per acre, whereas the Penn clay loam recorded a lime requirement of 1700 pounds per acre. In order to compare the soils on an equal reaction basis sufficient calcium carbonate was added to the Penn clay loam to bring it to the same acidity as the Norfolk sandy loam.

Part I

The Influence of Organic Matter on the Activity of Soil Fungi

Series I

It has generally been accepted that the addition of nitrogenous organic matter to the soil, especially that of vegetable origin containing a large proportion of readily decomposable carbohydrates, favors the growth of soil fungi. This fact often expresses itself by the presence of a large mycelial growth upon the surface of the soil in ammonification experiments. It becomes of interest therefore, to note the influence that different sources of organic matter would have upon the activity of soil fungi as measured by pure culture studies in sterilized soil.

Among the substances available for this purpose are many cereal grains, legume seeds, oil cakes, meals, straws, etc. Accordingly, a series was begun in which were used green rye, mature vetch, soybean meal, cottonseed meal, and for the purpose of comparison, dried blood, a material of animal origin. Cottonseed meal and dried blood constitute the major organic nitrogenous constituent of high grade fertilizers. A comparative study of their decomposition is, therefore, of much value as the rate of decay is not only an index of the availability of the material in question, but is also an index of the activity of the organism at hand.

The vetch and rye had approximately the same percentage of nitrogen, 3.42 per cent and 3.81 per cent, respectively. The percentage was high in the rye as it was cut when young. The cottonseed meal and soy-

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bean meal had 6.25 per cent and 6.52 per cent of nitrogen, respectively. The dried blood analyzed 12.54 per cent nitrogen.

The Fungi Studied

The fungi studied in this work were some of the more common species found in the soil. One or more of three groups of soil organisms were used. They were as follows: *Aspergillus niger* (van Tieghem); *Penicillium* sp. 10, *Rhizopus tritica, Zygorhynchus Vuilleminii* (Namyslowski) and *Trichoderma Koningi* (Oud.) (?).

Experiment I. The first fungus whose activity was studied was *Aspergillus niger.* This fungus has been the subject of experimentation by previous investigators, who have tried to measure its activity and importance to soil fertility by its power to fix atmospheric nitrogen. No one to the knowledge of the writer, has endeavored to measure its activity as a possible force in soil fertility by its power to transform organic nitrogenous materials into ammonia.

TABLE I

THE INFLUENCE OF ORGANIC MATTER UPON THE ACTIVITY OF ASPERGILLUS NIGER

Lab. No.	Source and amount of Nitrogen	Ammonia Accumulation				Inoculation
		Mg. N.	Mg. N.	Av. Mg. N.	Inc. Mg. N.	
1-2	155 mg. N. as Dried Blood....	2.84	2.68	2.74	....	None
3-4	155 mg. N. as Dried Blood....	3.71	4.34	4.02	1.78	1 c.c. Asp. nig.
5-6	155 mg. N. as Cottonseed Meal	3.54	3.37	3.45	....	None
7-8	155 mg. N. as Cottonseed Meal	10.18	11.14	10.61	7.16	1 c.c. Asp. nig.
9-10	155 mg. N. as Soybean Meal...	6.99	5.40	6.19	....	None
11-12	155 mg. N. as Soybean Meal...	15.36	11.52	13.44	7.23	1 c.c. Asp. nig.
13-14	155 mg. N. as Vetch.....	10.61	10.61	10.61	....	None
15-16	155 mg. N. as Vetch.....	10.66	11.14	10.92	.31	1 c.c. Asp. nig.
17-18	155 mg. N. as Rye.....	10.80	9.78	10.29	....	None
19-20	155 mg. N. as Rye.....	3.94	3.65	3.79	—6.50	1 c.c. Asp. nig.

The arrangement of the experiment, the amount of nitrogen recovered, and the spore count of the inoculum are given in Table I. This same arrangement follows throughout this investigation.

An examination of Table I shows us that this fungus is of relatively low activity with the conditions at hand. The organism had very little power to ammonify dried blood, only 1.78 mg. of nitrogen having accumulated as ammonia at the end of the incubation period. Soybean meal seems to be the most favorable material for the activity of this organism, as the largest accumulation of ammonia was noted with this material as the source of food. The amount transformed, however, was very low, being less than 5 per cent of the total nitrogen supplied. No accumulation of note was recorded where vetch was used as the ammonifiable material. Ammonia no doubt was formed, as a very heavy mycelial growth was noted. The nitrogen cleaved was probably transformed into the tis-

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sues of the fungus. This was likewise the case when green rye was used. With this material however, an actual loss of 6 mg. of accumulated ammonia was registered.

For the maximum activity of this fungus, organic matter of vegetable origin having a rather narrow carbon-nitrogen ratio seems to be of the most suitable source. Animal organic matter, even of a very narrow C-N ratio as illustrated with dried blood, is not an acceptable source of food for the best activity of this fungus.

Experiment II. The Aspergillaceae constitute a large group of soil fungi. Many of its members have been frequently isolated. A second experiment was, therefore, started in which another one of this group,—a Penicillium was used. The organism belongs to a group of soil Penicillia designated as "group 10" by Mr. S. A. Waksman (40) to whom the author is indebted for its indentification, as well as for the identification of *Rhizopus tritica.*

TABLE II

THE INFLUENCE OF ORGANIC MATTER UPON THE ACTIVITY OF PENICILLIUM SP. 10

Lab. No.	Source and amount of Nitrogen	Ammonia Accumulation				Inoculation
		Mg. N.	Mg. N.	Av. Mg. N.	Inc. Mg. N.	
21-22	155 mg. N. as Dried Blood....	2.68	2.80	2.74	....	None
23-24	155 mg. N. as Dried Blood....	6.09	6.84	6.46	3.72	1 c.c. Peni. sp. 10
25-26	155 mg. N. as Cottonseed Meal	3.54	3.37	3.45	....	None
27-28	155 mg. N. as Cottonseed Meal	13.63	13.03	13.33	9.88	1 c.c. Peni. sp. 10
29-30	155 mg. N. as Soybean Meal...	3.65	3.45	3.55	....	None
31-32	155 mg. N. as Soybean Meal...	11.30	9.74	10.52	6.47	1 c.c. Peni. sp. 10
33-34	155 mg. N. as Vetch.....	10.61	10.50	10.55	....	None
35-36	155 mg. N. as Vetch.....	12.55	13.25	12.90	2.35	1 c.c. Peni. sp. 10
37-38	155 mg. N. as Rye.....	9.36	9.36	9.36	....	None
39-40	155 mg. N. as Rye.....	13.36	14.08	13.72	4.36	1 c.c. Peni. sp. 10

Upon studying the data in Table II it is easily seen that this fungus is also a rather inactive organism as measured by the ammonia accumulated at the end of 7 days. Although a fair growth of mycelium was noted upon the dried blood cultures, only 3.72 mg. of nitrogen accumulated. Cottonseed meal proved to be the most readily ammonified. The green rye cultures had a very large mycelial growth. This may aid in explaining the low amount of ammonia accumulating from the degradation of this material. Vetch was a very poor source of organic matter for this fungus to act upon. Not only did this prove true as measured by ammonia acmulation; but also a survey of the culture flasks showed a very scant mycelial growth.

Experiment III. The third organism studied was *Rhizopus tritica.* At first it was thought that this organism was *Rhizopus nigricans.* When

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tested out, however, for the formation of a sexual stage as outlined by Blakeslee it did not form this characteristic. Further analysis showed it to be in all probability *Rhizopus tritica.*

Examining the yields of ammonia, in Table III, accumulated from the decomposition of the several organic nitrogenous materials it can be easily seen that *Rhizopus tritica* is a very active organism in the presence of all the organic materials. Soybean meal was the substance most readily decomposed by this fungus, 39.25 mg. or 25.30 per cent of the total nitrogen supplied, manifesting itself as ammonia at the end of the incubation period. Cottonseed meal was also a very suitable material for an enhanced activity of this organism. On considering the other nitrogenous materials it is patent that green rye and vetch were also quite easily decomposed by the fungus, 22.85 mg. and 16.51 mg. of nitrogen, respectively, having been transformed in 7 days.

TABLE III

THE INFLUENCE OF ORGANIC MATTER UPON THE ACTIVITY OF RHIZOPUS TRITICA

Lab. No.	Source and amount of Nitrogen	Ammonia Accumulation				Inoculation
		Mg. N.	Mg. N.	Av. Mg. N.	Inc. Mg. N.	
31-32	155 mg. N. as Dried Blood....	2.68	2.80	2.74	....	None
33-34	155 mg. N. as Dried Blood....	34.13	34.01	34.07	31.33	1 c.c. Rhiz. tritica
35-36	155 mg. N. as Cottonseed Meal	3.54	3.37	3.45	....	None
37-38	155 mg. N. as Cottonseed Meal	37.79	37.24	37.51	34.06	1 c.c. Rhiz. tritica
39-40	155 mg. N. as Soybean Meal..	3.65	3.45	3.55	....	None
41-42	155 mg. N. as Soybean Meal...	41.91	43.69	42.80	39.25	1 c.c. Rhiz. tritica
43-44	155 mg. N. as Vetch.....	11.24	9.78	10.51	....	None
45-46	155 mg. N. as Vetch.....	32.36	32.36	32.36	22.85	1 c.c. Rhiz. tritica
47-48	155 mg. N. as Rye.....	10.80	9.78	10.29	....	None
49-50	155 mg. N. as Rye.....	27.18	26.43	26.80	16.51	1 c.c. Rhiz. tritica

Quite in contrast to the two previously studied forms this fungus was very active with dried blood as a source of energy. The same general preference for organic matter of vegetable origin is at hand, however.

Mycelial growth was especially marked in all the culture flasks. This was noticeably true where cottonseed meal and green rye were used as the sources of ammonifiable material.

Experiment IV. The data recorded in Table IV give still further evidence as to the effect of organic matter upon the activity of soil fungi. The arrangement is the same as in the preceding work. The fungus studied was *Zygorhynchus Vuilleminii* (Namyslowski). In striking contrast to *Rhizopus tritica* and correlating more closely with the members of the Aspergillaceae studied, low activity was evidenced in the presence of dried blood. As a very active organism in the presence of organic matter of vegetable origin, this fungus compares very favorably with *Rhizopus tritica.* Soybean meal and cottonseed meal seem to regulate a

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similar activity when placed in the presence of this fungus, soybean meal inducing a somewhat greater activity. Vetch and rye are also materials suitable for a marked activity of this organism. With the conditions at hand vegetable organic matter was ammonified to the greatest extent. Dried blood was not a good source of energy for the best activity of this fungus.

TABLE IV

THE INFLUENCE OF ORGANIC MATTER UPON THE ACTIVITY OF ZYGORHYNCHUS VUILLEMINII

Lab. No.	Source and amount of Nitrogen	Ammonia Accumulation				Inoculation
		Mg. N.	Mg. N	Av. Mg. N.	Inc. Mg. N.	
61-62	155 mg. N. as Dried Blood....	2.68	2.80	2.74	....	None
63-64	155 mg. N. as Dried Blood....	8.67	8.97	8.82	6.08	1 c.c. Zyg. Vuil.
65-66	155 mg. N. as Cottonseed Meal	3.54	3.40	3.47	....	None
67-68	155 mg. N. as Cottonseed Meal	27.71	28.73	28.22	24.75	1 c.c. Zyg. Vuil.
69-70	155 mg. N. as Soybean Meal...	3.65	3.50	3.57	....	None
71-72	155 mg. N. as Soybean Meal ..	30.96	31.08	31.07	27.45	1 c.c. Zyg. Vuil.
73-74	155 mg. N. as Vetch.....	11.24	9.78	10.51	....	None
75-76	155 mg. N. as Vetch.....	27.27	27.29	27.28	16.77	1 c.c. Zyg. Vuil.
77-78	155 mg. N. as Rye.....	9.36	9.36	9.36	....	None
79-80	155 mg. N. as Rye.....	24.50	23.31	23.90	14.54	1 c.c. Zyg. Vuil.

Experiment V. Another group of soil fungi not as yet considered are the Moniliaceae. McLean and Wilson (26) have shown in their work that these organisms were the most efficient ammonifiers of any of the fungi which they studied. One of the Trichodermae which at the present writing seems to be *Trichoderma Koningi* (Oud.), was used. A casual glance at Table V will at once convince one that this organism is exceedingly active in the presence of all five sources of organic matter. In fact it is the most active organism thus far met with and is also in confirmation of the work of McLean and Wilson (26).

TABLE V

THE INFLUENCE OF ORGANIC MATTER UPON THE ACTIVITY OF TRICHODERMA KONINGI

Lab. No.	Source and amount of Nitrogen	Ammonia Accumulation				Inoculation
		Mg. N.	Mg. N.	Av. Mg. N.	Inc. Mg. N.	
81-82	155 mg. N. as Dried Blood....	2.68	2.80	2.74	....	None
83-84	155 mg. N. as Dried Blood....	40.59	41.57	41.08	39.34	1 c.c. Trich. Kon.
85-86	155 mg. N. as Cottonseed Meal	3.54	3.37	3.45	....	None
87-88	155 mg. N. as Cottonseed Meal	42.63	42.33	42.48	39.03	1 c.c. Trich. Kon.
89-90	155 mg. N. as Soybean Meal...	3.65	3.45	3.55	....	None
91-92	155 mg. N. as Soybean Meal...	46.66	45.70	46.18	42.63	1 c.c. Trich. Kon.
93-94	155 mg. N. as Vetch.....	10.61	10.61	10.61	....	None
95-96	155 mg. N. as Vetch.....	30.23	29.23	29.73	19.12	1 c.c. Trich. Kon.
97-98	155 mg. N. as Rye.....	13.63	13.03	13.33	....	None
99-100	155 mg. N. as Rye.....	14.69	17.74	16.21	2.89	1 c.c. Trich. Kon.

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Soybean meal was ammonified to the greatest extent. Not only does this hold true in this particular experiment, but it likewise holds true in comparison with all previous experimentation. Of the 155 mg. of nitrogen supplied, 42.63 mg. accumulated as ammonia in 7 days. Cottonseed meal and dried blood seemed to be identical in regulating the activity of this fungus. With rye, on the other hand, very little ammonia accumulated. The activity of the fungus in the presence of this material seemed to be mainly one of building tissue, i.e. consumption of any cleared am-

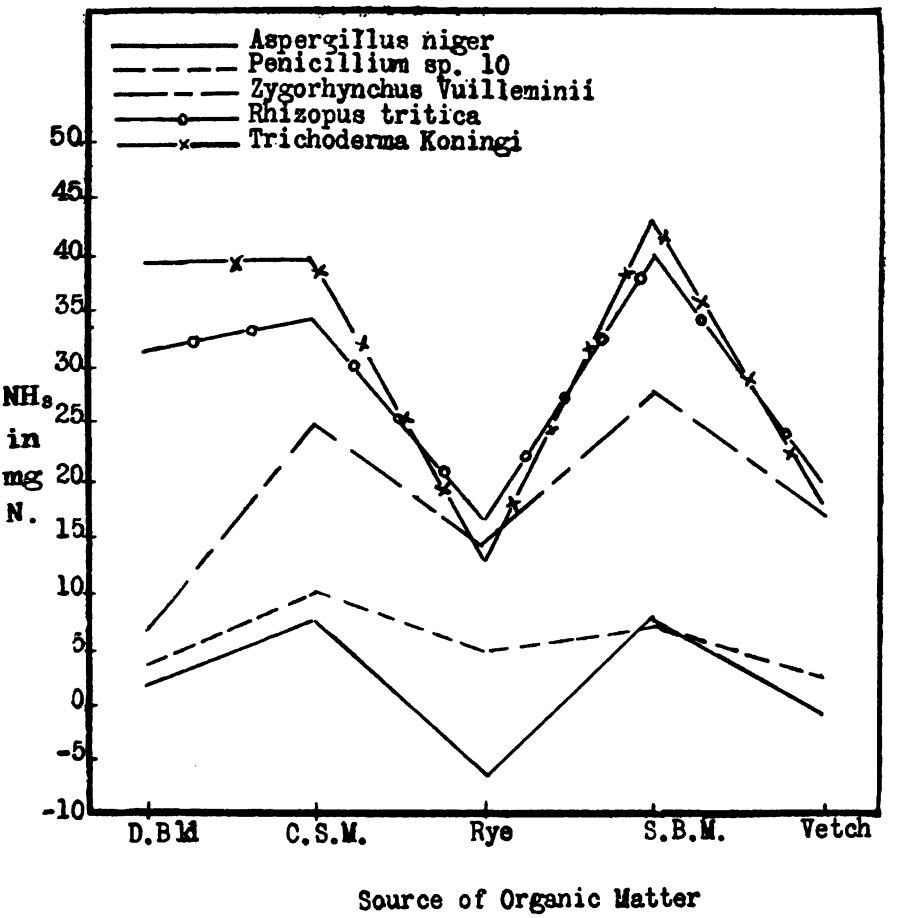

Fig. 1.—Diagram showing the influence of organic matter on the activity of soil fungi.

monia. Vetch was transformed to an appreciable extent, 19.12 mg. of nitrogen accumulating at the end of 7 days. This organism was just as active in the presence of material of vegetable nature as it was in the presence of material of animal nature.

This group of organisms is very closely related to the Penicillia. It is of striking interest to note the wide differences in their activities. The greatest activity, as measured by ammonia accumulation, recorded by the

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species of *Penicillium* at hand was 9.88 mg. of nitrogen, whereas the largest accumulation with *Trichoderma Koningi* was 42.63 mg.

The above data would seem to indicate that activity of soil fungi, as measured by their power to decompose organic matter in sterilized soil, would vary with the quality of the material at hand. Materials of vegetable origin were more suitable for the maximum activity of these organisms. This is noticeable where materials of a rather narrow C-N ration are present. Dried blood, however, was quite favorable as a source of energy for the activity of *Rhizopus tritica* and *Trichoderma Koningi.*

A graphic representation of the activities of these organisms in this series is shown in figure 1.

The high activity of the organisms in the presence of the different organic materials is probably due in no small measure to the mechanical condition of the soil. To throw some light upon this phase, as well as to serve as a check upon Series I a second series was started in which a "heavier" clay loam soil was used. Otherwise, the following series is an exact duplication of Series I.

Series II

Experiment VI. That the aeration of the clay loam soil is in all probability inimical to the activity of soil fungi, even though the same source of nitrogen is supplied for the ammonification process, is evident

TABLE VI

THE INFLUENCE OF ORGANIC MATTER UPON THE ACTIVITY OF ASPERGILLUS NIGER

Lab. No.	Source and amount of Nitrogen	Ammonia Accumulation				Inoculation
		Mg. N.	Mg. N.	Av. Mg. N.	Inc. Mg. N.	
101-102	155 mg. N. as Dried Blood....	2.77	2.48	2.62	....	None
103-104	155 mg. N. as Dried Blood....	3.21	2.56	2.88	.26	1 c.c. Asp. niger
105-106	155 mg. N. as Cottonseed Meal	4.38	4.00	4.19	....	None
107-108	155 mg. N. as Cottonseed Meal	7.19	6.39	6.79	2.60	1 c.c. Asp. niger
109-110	155 mg. N. as Soybean Meal..	2.63	2.92	2.77	....	None
111-112	155 mg. N. as Soybean Meal..	5.35	....	5.35	2.58	1 c.c. Asp. niger
113-114	155 mg. N. as Vetch.....	9.34	11.51	10.42	....	None
115-116	155 mg. N. as Vetch.....	9.63	9.62	9.62	—.80	1 c.c. Asp. niger
117-118	155 mg. N. as Rye.....	9.20	9.78	9.49	....	None
119-120	155 mg. N. as Rye.....	7.44	7.30	7.37	—2.12	1 c.c. Asp. niger

from a casual glance at the accumulated data. This fact is especially brought out when we consider the activities of *Aspergillus niger* in this series. Table VI records the data. No activity manifested itself as accumulated ammonia when dried blood was used as the source of organic matter. No visible growth of mycelium was noticed on the surface of the soil or within the soil strata. Growth of the organism was noted on all of the cultures which contained the vegetable sources of organic matter. Only two of them, cottonseed meal and soybean meal registered as

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having been decomposed to any appreciable extent. It is thus evident that this organism is quite inactive with the conditions at hand in this type of soil.

Although, as was previously mentioned, growth of the organism was noted upon the vetch and rye cultures, no measurable decomposition of these substances took place. In fact, a loss of nitrogen was recorded in the two instances. As was brought out in Series I, vegetable organic matter of high quality is necessary for the best activity of this organism.

Experiment VII. A somewhat identical state of affairs presents itself in the studies with the species of *Penicillium* in this series. Here however, some activity is to be noted. In the presence of dried blood, soybean meal and cottonseed meal again proved to be adaptable sources of organic matter for the greatest activity of this organism. Loss of accu-

TABLE VII

THE INFLUENCE OF ORGANIC MATTER UPON THE ACTIVITY OF PENICILLIUM SP. 10

Lab. No.	Source and amount of Nitrogen	Ammonia Accumulation				Inoculation
		Mg. N.	Mg. N.	Av. Mg. N.	Inc. Mg. N.	
121-122	155 mg. N. as Dried Blood....	2.77	2.48	2.62	....	None
123-124	155 mg. N. as Dried Blood....	4.81	3.65	4.23	1.61	1c.c. Peni. sp. 10
125-126	155 mg. N. as Cottonseed Meal	4.38	4.00	4.19	....	None
127-128	155 mg. N. as Cottonseed Meal	6.07	6.00	6.03	1.84	1 c.c. Peni. sp. 10
129-130	155 mg. N. as Soybean Meal...	2.63	2.92	2.77	....	None
131-132	155 mg. N. as Soybean Meal...	4.81	5.98	5.39	2.62	1 c.c. Peni. sp. 10
133-134	155 mg. N. as Vetch.....	9.24	11.51	10.37	....	None
135-136	155 mg. N. as Vetch.....	6.08	7.73	6.90	—3.47	1 c.c. Peni. sp. 10
137-138	155 mg. N. as Rye.....	9.20	9.78	9.49	....	None
139-140	155 mg. N. as Rye.....	7.84	9.03	8.43	—1.06	1 c.c. Peni. sp. 10

mulated ammonia is again noted where vetch and rye were used as the source of organic matter. This time, however, the greatest loss was recorded where vetch was used. In the previous series the greatest loss was noted where rye was employed.

Experiment VIII. Rhizopus tritica also seems to be affected by the change in the type of soil. Table VIII records the data to show the activity of this organism in the clay loam soil.

Soybean meal was again the best source of organic matter for the maximum activity of this organism. Dried blood was ammonified to a greater extent in this series than was cottonseed meal. In Series I the reverse was true. Vetch was also ammonified to a large extent, 19.22 mg. of nitrogen having accumulated at the end of the incubation period. In comparison with Series I the activity of the fungus in the presence of rye was also somewhat lessened.

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TABLE VIII

THE INFLUENCE OF ORGANIC MATTER UPON THE ACTIVITY OF RHIZOPUS TRITICA

Lab. No.	Source and amount of Nitrogen	Ammonia Accumulation				Inoculation
		Mg. N.	Mg. N.	Av. Mg. N.	Inc. Mg. N.	
141-142	155 mg. N. as Dried Blood....	6.62	5.43	6.03	....	None
143-144	155 mg. N. as Dried Blood....	21.96	23.14	22.55	16.52	1 c.c. Rhiz. tritica
145-146	155 mg. N. as Cottonseed Meal	6.54	6.24	6.39	....	None
147-148	155 mg. N. as Cottonseed Meal	17.06	19.22	18.14	11.75	1 c.c. Rhiz. tritica
149-150	155 mg. N. as Soybean Meal...	4.51	4.48	4.49	....	None
151-152	155 mg. N. as Soybean Meal...	26.83	25.65	26.24	21.75	1 c.c. Rhiz. tritica
153-154	155 mg. N. as Vetch.....	9.34	11.51	10.42	....	None
155-156	155 mg. N. as Vetch.....	29.60	29.68	29.64	19.22	1 c.c. Rhiz. tritica
157-158	155 mg. N. as Rye.....	9.99	9.63	9.81	....	None
159-160	155 mg. N. as Rye.....	22.07	22.27	22.17	12.36	1 c.c. Rhiz. tritica

Experiment IX. Table IX records the activity of *Zygorhynchus Vuilleminii* in this "heavier" soil type. A glance will point out to the reader that this fungus is not nearly as susceptible to change in soil conditions as were those fungi previously studied. Although the ammonia accumulation is somewhat lessened in comparison with Series I, it is in no case in excess of 6 per cent and in some cases less than 2 per cent. Soybean meal, as in the previous series was best ammonified. Vetch

TABLE IX

THE INFLUENCE OF ORGANIC MATTER UPON THE ACTIVITY OF ZYGORHYNCHUS VUILLEMINII

Lab. No.	Source and amount of Nitrogen	Ammonia Accumulation				Inoculation
		Mg. N.	Mg. N.	Av. Mg. N.	Inc. Mg. N.	
161-162	155 mg. N. as Dried Blood....	6.62	5.43	6.03	....	None
163-164	155 mg. N. as Dried Blood....	11.44	11.04	11.24	5.20	1 c.c. Zyg. Vuil.
165-166	155 mg. N. as Cottonseed Meal	6.54	6.24	6.39	....	None
167-168	155 mg. N. as Cottonseed Meal	25.30	24.70	25.00	18.61	1 c.c. Zyg. Vuil.
169-170	155 mg. N. as Soybean Meal...	9.90	9.63	9.81	....	None
171-172	155 mg. N. as Soybean Meal...	22.37	22.30	22.33	12.52	1 c.c. Zyg. Vuil.
173-174	155 mg. N. as Vetch.....	6.60	6.83	6.71	....	None
175-176	155 mg. N. as Vetch.....	27.68	28.88	28.23	21.59	1 c.c. Zyg. Vuil.
177-178	155 mg. N. as Rye.....	9.34	11.51	10.42	....	None
179-180	155 mg. N. as Rye.....	25.60	24.60	25.10	14.68	1 c.c. Zyg. Vuil.

was very effectively decomposed, 14.68 mg. of nitrogen having accumulated at the end of 7 days. This is not in excess of the ammonification of cottonseed meal and rye which were more effectively cleaved in the previous series by this organism. Dried blood was again poorly ammonified. It was, however, as well ammonified in this series as in Series I.

Experiment X. Turning our attention to the activities of *Trichoderma Koningi* in this series we find an exceptionally high ammonification of all the organic nitrogenous materials.

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A casual glance at the ammonia accumulation shows us that soybean meal was ammonified by this fungus in a perfectly amazing manner. 70.75 mg. or over 45 per cent of the total supplied nitrogen was transformed and accumulated as ammonia in 7 days. This large ammonification is likewise apparent when we observe the figures showing the ammonia accumulation from the decomposition of dried blood and cotton-seed meal. Over 50 mg. of nitrogen or 33 per cent of the total supplied nitrogen were transformed into ammonia in 7 days. Twenty per cent of the vetch was likewise transformed into ammonia during the course of the incubation period.

TABLE X

THE INFLUENCE OF ORGANIC MATTER UPON THE ACTIVITY OF TRICHODERMA KONINGI

Lab. No.	Source and amount of Nitrogen	Ammonia Accumulation				Inoculation
		Mg. N.	Mg. N.	Av. Mg. N.	Inc. Mg. N.	
181-182	155 mg. N. as Dried Blood....	6.62	5.43	6.03	....	None
183-184	155 mg. N. as Dried Blood....	57.92	55.31	56.61	50.58	1 c.c. Trich. Kon.
185-186	155 mg. N. as Cottonseed Meal	6.54	6.24	6.39	....	None
187-188	155 mg. N. as Cottonseed Meal	56.22	54.07	55.14	48.75	1 c.c. Trich. Kon.
189-190	155 mg. N. as Soybean Meal...	6.60	6.83	6.70	....	None
191-192	155 mg. N. as Soybean Meal...	74.73	80.40	77.56	70.75	1 c.c. Trich. Kon.
193-194	155 mg. N. as Vetch.....	5.38	5.84	5.61	....	None
195-196	155 mg. N. as Vetch.....	41.15	35.80	38.47	32.88	1 c.c. Trich. Kon.
197-198	155 mg. N. as Rye.....	9.97	9.63	9.80	....	None
199-200	155 mg. N. as Rye.....	25.76	25.65	25.70	15.90	1 c.c. Trich. Kon.

In order to compare the relative activities of these organisms in the presence of the various kinds of organic matter in the different types of soil, the data from Series I and II have been summarized in Table A and also for Series II a graphic representation is given in figure 2. Only the ammonia accumulations after deducting the blank determinations are given. The spore count of the respective inoculations is shown in Table B so that a more careful comparison may be accomplished. It has been shown by Kopeloff (15) in a recent publication that approximately equivalent numbers of spores must be used for inoculation purpose in order to obtain comparable results.

The spore count of the *Aspergillus niger, Penicillium* sp., *Rhizopus tritica* and *Zygorhynchus Vuilleminii* cultures in both series are close enough for a just comparison of the activities of these organisms. Unfortunately the spore counts with *Trichoderma Koningi* are not so close as to warrant so intimate a comparison.

A survey of the data in Table A shows beyond doubt that vegetable organic matter of a fairly narrow C-N ratio is very well adapted to the best activities of the soil fungi studied. Dried blood proved to be an acceptable source of energy for the activity of the organisms in only a few instances, viz., with *Rhizopus tritica* and *Trichoderma Koningi*. This is

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true even with a change in environment resulting from the substitution of a second type of soil. Greater activity is to be noted in Series I than in Series II. This is due in a large measure, perhaps, to the greater aeration in Series I.

TABLE A

NITROGEN (MG.) TRANSFORMED FROM ALL SOURCES OF ORGANIC MATTER IN BOTH SOILS

Name of Organism	Sandy Soil					Clay Soil				
	D. Blood	C. S. M.	S. B. M.	Vetch	Rye	D. Blood	C. S. M.	S. B. M.	Vetch	Rye
Aspergillus niger	1.78	7.16	8.23	+.31	-6.50	.26	2.60	2.58	—.80	-2.12
Penicillium, sp. 10	3.72	9.88	6.97	2.35	4.36	1.61	1.84	2.62	-3.47	-1.06
Rhizopus tritica	31.33	34.06	39.25	22.85	16.51	16.52	11.75	21.75	19.22	12.36
Zygorhynchus Vuilleminii ...	6.08	24.75	27.45	16.77	14.54	5.20	18.61	21.59	14.68	12.52
Trichoderma Koningi	39.34	39.03	42.63	19.12	12.89	50.58	48.75	70.75	32.88	16.90

Considering more closely the activity of the various organisms we note that *Trichoderma Koningi* seems to be the most active organism at hand. The greatest activity of this organism was manifested in the presence of soybean meal. This is true in both types of soil. In fact greater activity was noted in Series II than in Series I indicating that the larger inoculation of spores in this series was responsible for the greater decomposition, or perhaps the bettered chemical environment may be an enhancing factor.

TABLE B

NUMBER OF SPORES PER C.C. OF INOCULATING MATERIAL

Name of Organism	Sandy Soil	Clay Soil
Aspergillus niger	209,400	180,000
Penicillium, sp. 10	392,000	400,000
Rhizopus tritica	482,000	464,000
Zygorhynchus Vuilleminii	55,000	56,400
Trichoderma Koningi	192,000	286,000

The activity of this fungus in the presence of dried blood and cottonseed meal is about the same in both types of soil. A very marked activity was also to be noted in the flasks containing vetch and rye as the ammonifiable material.

Rhizopus tritica also enjoys marked activity in soil with dried blood as the source of energy. Its activity is somewhat curtailed, however, in Series II. This organism also exhibits the greatest activity where soybean meal is at hand. Dried blood and cottonseed meal were decomposed to about the same extent. This fungus assumed the greatest activity of all in the presence of vetch and rye in Series I, whereas, *Trichoderma Koningi* attained this rank in Series II.

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Zygorhynchus Vuilleminii exhibited very little activity when dried blood was the material to be decomposed. It has been shown by Kopeloff (16) that a very high acidity is necessary for the maximum activity of this organism in the presence of dried blood. This investigator gives data to show a 500 per cent increase in the decomposing power of this organism by raising the acidity of the soil from neutral to an acidity equal

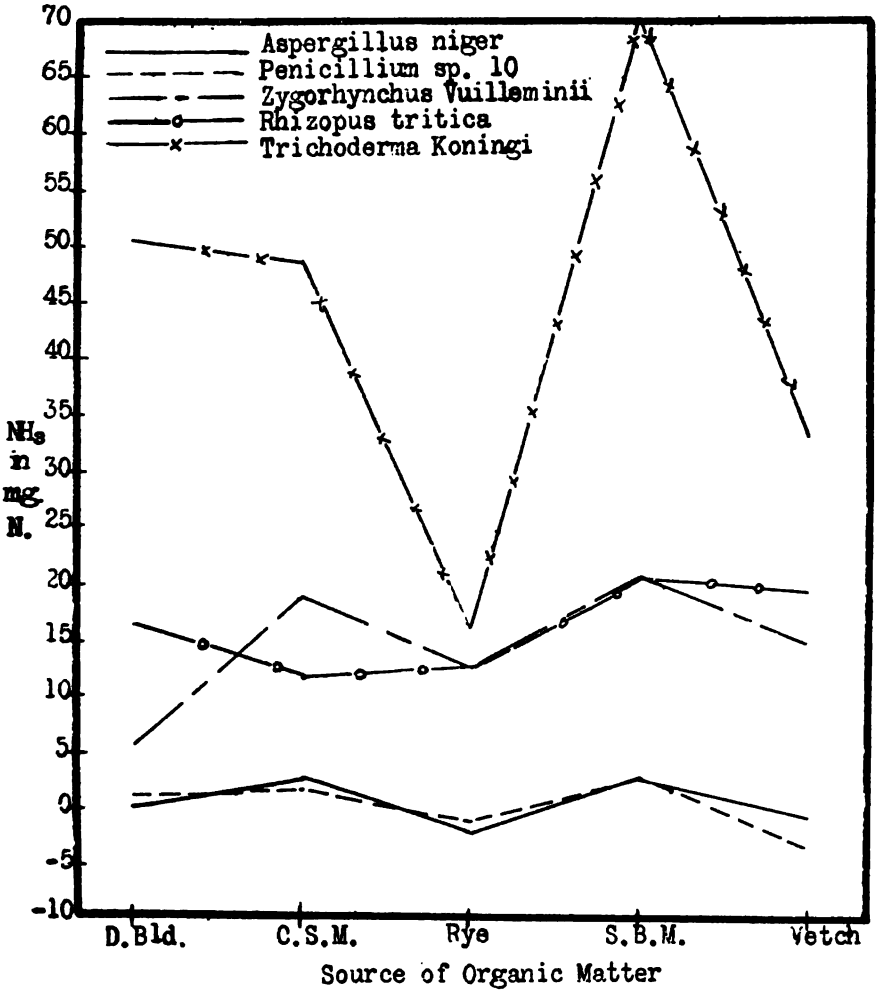

Fig. 2.—Diagram showing the influence of organic matter on the activities of soil fungi.

to 2000 pounds of calcium oxide per acre. This organism was also quite active in the presence of soybean meal and cottonseed meal, a few mg. difference being recorded, however, between the action of the two materials. This fungus was also a very efficient ammonifier of rye and vetch, but little depression in the activities of this organism is to be noted with a change in soil type with these sources of energy.

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The two members of the Aspergillaceae were the most drastically affected by the change of soil conditions. Of the two, the species of *Penicillium* seems to be the active organism. Soybean meal and cottonseed meal were both suitable for a measureable activity of these organisms, however. Dried blood was not conducive to the activity of these organisms with the conditions at hand. Kopeloff (16) has, however, shown that *Penicillium* sp. 10 responds very well to a high acid reaction in decomposing this material.

Vetch and rye seemed to promote excessive growth of the organism at the expense of ammonia accumulation.

It is difficult to say that any one organism constantly stands as the most active regardless of soil and ammonifiable material supplied. In other words, it appears, viewing the activity of these organisms from the standpoint of pure cultures, that every organism will do best with a definite combination of soil and organic matter, the quality of the organic matter and the mechanical composition of the soil being important factors. There is, however, one organism which nearly approaches such a description, viz., *Trichoderma Koningi.*

In attempting to explain the various differences in the ammonifiability of the different nitrogenous materials the C-N ration is no doubt an important item.

The reason that soybean meal proved superior to all other materials as a source of food for the activity of the organisms in question is no doubt due to the high percentage of oil and sucrose in the sample. Analyses showed that the amount of oil was approximately 20 per cent, whereas sucrose was present to the amount of 4 per cent.

Cottonseed meal, with approximately the same percentage of nitrogen was ammonified next in order, considering materials of vegetable origin. This material had lost a large portion of its oil and consequently was not available for plant growth. The lower ammonification and subsequent lessened activity is accountable, in a measure, to this fact.

Dried blood, although having a much narrower C-N ratio than any of the other materials was less acceptable, in the majority of cases to the activity of these organisms studied, materials of a much wider C-N ratio being more conducive to enhanced activity.

Considering the two other sources of organic matter, a much wider C-N ratio is present. Of the two, vetch was in general, better suited to fungus activity than rye. The vetch, being a legume, would tend to have more fatty and oily substances than rye. In a measure, therefore, the better activity of the organisms in the presence of this material is accounted for. In all the experiments where these materials were used the organisms exhibited enormous mycelial growths. As this condition must necessarily demand the consumption of food, it is thought that the de-

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graded protein was consumed by the fungi in forming the large mycelial growth present.

It is difficult to say just why vetch and sometimes rye should show an actual loss of accumulated ammonia unless the above phenomena take place.

A comparison of the data submitted herein, with that recently published by Lipman (19) of California, working with bacteria indicates that under suitable conditions some fungi are more efficient than bacteria in decomposing nitrogenous organic matter.

Summary of Part I

Results are given which show that:

1. Marked differences were observed in the activity of soil fungi in pure culture in sterilized soil.
2. The soil used was of two types: a sandy loam and a clay loam.
3. Five sources of organic matter were employed in both types of soil, four of vegetable origin and one of animal origin.
4. Organic matter of vegetable origin was in the main more suitable for the highest activity of the soil fungi studied.
5. The quality of the organic matter has much to do with the activity of soil fungi.
6. The most active organism was *Trichoderma Koningi;* the least active *Aspergillus niger.*
7. Marked differences were noticed in the activity of closely related groups of organisms in the presence of the same source of organic matter.
8. Some fungi seem to be more efficient than soil bacteria in decomposing nitrogenous organic matter.
9. Aeration seems to be a controlling factor in the activity of soil fungi.
10. Materials of a rather wide C-N ratio are fairly acceptable for the activity of the organisms noted.

Part II

The Influence of the Mechanical and Chemical Composition of the Soil

In order to obtain additional data concerning the activity of these organisms as influenced by the chemical and mechanical composition of the soil the following experiments were made.

One-hundred-gm. portions of the "heavy" clay loam used in the previous experimentation were diluted with pure quartz sand in varying proportions as indicated in the tables to follow. One hundred fifty-five mg. of nitrogen as cottonseed meal were added as ammonifiable material to each portion, the whole thoroughly mixed, the proper moisture added and sterilized in the autoclave as before. The inoculation, incubation and subsequent analyses of the degraded protein was performed as usual.

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In order to test the influence of the chemical factors with regard to the activities of soil fungi, varying amounts of the respective chemicals were added to 100-gm. portions of the Penn clay loam. The soil and organic matter were first thoroughly mixed and the salts, when soluble, added in solution to afford better mixing. Where this was not feasible, the chemicals were added in the dry form and thoroughly mixed with the soil.

The chemicals employed were nitrate of soda, chloride of potash, and acid phosphate. The first two were applied in amounts equivalent to 100, 300, and 500 pounds per acre on a 2,700,000-pound basis. The acid phosphate was applied at the rate of 800, 1000 and 1200 pounds per acre. The object being to conform to field conditions as closely as possible.

The fungi used were *Aspergillus niger, Rhizopus tritica, Zygorhynchus Vuilleminii,* and *Trichoderma Koningi.*

Experiment XI. The results obtained with *Aspergillus niger* are given in Table XI.

TABLE XI

THE EFFECT OF THE MECHANICAL COMPOSITION OF THE SOIL UPON THE ACTIVITY OF ASPERGILLUS NIGER

Lab. No.	Soil Mixture	Ammonia Accumulation			Increase over no treatment
		Mg. N.	Mg. N.	Av. mg. N.	
201-202	100 gm. loam	9.45	7.20	8.32	....
203-204	90 gm. loam + 10 gm. SiO_2	7.20	9.90	8.55	.23
205-206	80 gm. loam + 20 gm. SiO_2	10.21	12.62	11.41	3.09
207-208	70 gm. loam + 30 gm. SiO_2	15.16	16.12	15.64	7.32
209-210	60 gm. loam + 40 gm. SiO_2	12.98	13.00	12.99	4.67
211-212	50 gm. loam + 50 gm. SiO_2	14.60	16.15	15.38	7.06
213-214	40 gm. loam + 60 gm. SiO_2	20.13	19.11	19.62	11.30
215-216	30 gm. loam + 70 gm. SiO_2	23.66	20.78	22.22	13.90
217-218	20 gm. loam + 80 gm. SiO_2	25.44	26.31	25.87	17.55
219-220	10 gm. loam + 90 gm. SiO_2	36.87	37.31	37.09	28.77

It is apparent from the results here tabulated that this fungus is highly influenced by the increased aeration resulting from the additions of the quartz sand. In the main, increased activity goes hand in hand with increased oxygen pressure. This is quite remarkable when one considers that the increased proportions of sand are in the same degree depressing the beneficial influences of the chemical composition of the soil.

The beneficial effect of increased aeration commences to show itself when 20 per cent of sand has been added to the soil and from here on, with the exception of soil portions 209-210, a gradual increase in accumulated ammonia was recorded. The depression in the above mentioned instance is due, in all probability, to the inefficient mixing of sand and soil.

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Experiment XII. The influence which the chemical composition of the soil has upon the activity of this fungus seems to be quite negligible. Table XII records the experimentation. In order to bring out more forcefully the influences of the chemicals applied, the incubation period was lengthened to 12 days. The reader can at once see that any activity manifested, either of a depressed or an enhanced nature, is well within experimental error.

TABLE XII

THE EFFECT OF THE CHEMICAL COMPOSITION OF THE SOIL UPON THE ACTIVITY OF ASPERGILLUS NIGER

Lab. No.	Soil Treatment	Ammonia Accumulation			Increase over no treatment
		Mg. N.	Mg. N.	Av. mg. N.	
221-222	100 lbs. $NaNO_3$	16.10	15.60	15.85	.....
223-224	300 lbs. $NaNO_3$	16.60	16.50	16.55	+.70
225-226	500 lbs. $NaNO_3$	14.30	16.40	15.35	—.50
227-228	100 lbs. KCl	14.40	16.90	15.65	—.20
229-230	300 lbs. KCl	15.60	16.00	15.80	—.05
231-232	500 lbs. KCl	13.30	14.90	14.20	—1.65
233-234	800 lbs. Acid Phosphate	14.20	.....	14.20	—1.65
235-236	1000 lbs. Acid Phosphate	16.70	14.30	15.50	—.35
237-238	1200 lbs. Acid Phosphate	16.10	15.60	15.85	.....
239-240	No treatment	15.40	16.30	15.85	.....

In view of the fact that this organism was so very responsive to a bettered oxygen supply at the expense of a depressed chemical environment the above results do not appear phenomenal.

Experiment XIII. The activity of *Rhizopus tritica* in the presence of bettered oxygen conditions is recorded in Table XIII. Increased ac-

TABLE XIII

THE EFFECT OF THE MECHANICAL COMPOSITION OF THE SOIL UPON THE ACTIVITY OF RHIZOPUS TRITICA

Lab. No.	Soil Mixture	Ammonia Accumulation			Increase over no treatment
		Mg. N.	Mg. N.	Av. mg. N.	
281-282	100 gm. loam	24.78	24.08	24.43	.....
283-284	90 gm. loam + 10 gm. SiO_2	30.10	28.70	29.40	4.97
285-286	80 gm. loam + 20 gm. SiO_2	30.24	31.22	30.73	6.30
287-288	70 gm. loam + 30 gm. SiO_2	30.80	31.92	31.36	6.93
289-290	60 gm. loam + 40 gm. SiO_2	32.62	31.92	32.27	7.84
291-292	50 gm. loam + 50 gm. SiO_2	33.46	34.44	33.95	9.52
293-294	40 gm. loam + 60 gm. SiO_2	36.54	35.14	35.84	11.41
295-296	30 gm. loam + 70 gm. SiO_2	33.60	37.80	35.70	11.27
297-298	20 gm. loam + 80 gm. SiO_2	34.34	37.80	36.07	11.64
299-300	10 gm. loam + 90 gm. SiO_2	32.62	33.76	33.19	8.76

tivity commenced to exhibit itself when the soil was diluted with 10 per cent of sand. As was the case with *Aspergillus niger,* increased activity went hand in hand with increased oxygen supply. While the succeeding

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increments of sand did not cause proportionally as enhanced an activity as the initial dilution, nevertheless the increased oxygen pressure was accompanied by a gradual increased activity. It is interesting to note that this activity is constant in spite of the poorer chemical composition of the medium due to the dilution of the soil. That this organism also responds to a better chemical environment is also evident from the data tabulated below.

Experiment XIV. It is interesting to note that all three chemicals in all three applications stimulate activity to about the same degree. Where nitrate equivalent to 100 pounds of nitrate of soda was added a 25 per cent increased activity is to be noted. Higher applications do not cause any depressed or stimulated action.

TABLE XIV

THE EFFECT OF THE CHEMICAL COMPOSITION OF THE SOIL UPON THE ACTIVITY OF RHIZOPUS TRITICA

Lab. No.	Soil Treatment	Ammonia Accumulation			Increase over no treatment
		Mg. N.	Mg. N.	Av. mg. N.	
301-302	100 lbs. $NaNO_3$	30.66	31.55	31.10	6.67
303-304	300 lbs. $NaNO_3$	31.55	30.66	31.10	6.67
305-306	500 lbs. $NaNO_3$	30.15	30.80	30.47	6.04
307-308	100 lbs. KCl	29.68	31.72	30.45	6.02
309-310	300 lbs. KCl	30.15	30.80	30.47	6.04
311-312	500 lbs. KCl	32.63	30.00	31.31	6.88
313-314	800 lbs. Acid Phosphate	31.92	32.62	32.27	7.84
315-316	1000 lbs. Acid Phosphate	30.38	31.22	30.80	6.37
317-318	1200 lbs. Acid Phosphate	31.26	lost	31.26	6.83
319-320	No treatment	24.78	24.08	24.43	.....

This was likewise the case where potash was applied. The increased activity was about the same as noted with the nitrate salt.

Acid phosphate gave the highest stimulation of all three chemicals when applied at the rate of 800 pounds per acre, a 30 per cent increase resulting. Larger quantities had little influence either in enhancing or depressing the activity of this fungus.

A resumé of the experimentation above reported shows us that this organism at hand is quite responsive to an increased oxygen supply even at the expense of a lowered chemical environment. The fungus, however, also responds very well to a bettered chemical environment. Acid phosphate produces the greatest increase in activity, whereas, nitrate of soda and chloride of potash exert a somewhat identical stimulation. A comparison of the chemical and physical factors indicates, as a whole, that the physical factors are more influential than the chemical factors.

Experiment XV. The organisms previously studied have all shown response to a better oxygen supply. The data in Table XV on the other hand seem to indicate that *Zygorhynchus Vuilleminii* is not very responsive to an increased oxygen supply.

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It cannot be stated that this organism is not absolutely irresponsive to an increased oxygen supply as a slightly enhanced effect is to be seen in nearly every case. No marked action as measured by the accumulation of ammonia was noted, however. The greatest activity was noted in almost pure sand, i. e., soil portions 339 and 340. In some cases a decrease is to be noted which may be ascribed to several reasons, i. e., inefficient mixing, inoculation, etc.

TABLE XV

THE EFFECT OF THE MECHANICAL COMPOSITION OF THE SOIL UPON THE ACTIVITY OF ZYGORHYNCHUS VUILLEMINII

Lab. No.	Soil Mixture	Ammonia Accumulation			Increase over no treatment
		Mg. N.	Mg. N.	Av. mg. N.	
321-322	100 gm. loam	28.00	28.90	28.45	.....
323-324	90 gm. loam + 10 gm. SiO_2	30.30	29.80	30.05	1.70
325-326	80 gm. loam + 20 gm. SiO_2	29.40	28.80	28.60	.15
327-328	70 gm. loam + 30 gm. SiO_2	28.00	31.70	29.85	1.40
329-330	60 gm. loam + 40 gm. SiO_2	28.90	30.90	29.90	1.45
331-332	50 gm. loam + 50 gm. SiO_2	30.10	27.90	29.00	.55
333-334	40 gm. loam + 60 gm. SiO_2	24.60	.....	24.60	—3.85
335-336	30 gm. loam + 70 gm. SiO_2	32.00	29.75	30.87	2.42
337-338	20 gm. loam + 80 gm. SiO_2	25.80	25.40	25.60	—2.85
339-340	10 gm. loam + 90 gm. SiO_2	34.40	29.10	31.75	3.30

Experiment XVI. Although this fungus does not seem to be responsive to an increased oxygen supply its activity in the presence of various fertilizing elements is quite pronounced as can be seen by consulting Table XVI. Increased quantities of nitrates give a gradual stimulating influence. Where nitrates were added equivalent to 100 pounds of nitrate of soda an increased accumulation of 0.7 mg. of nitrogen is to be noted. Three hundred pounds increased the accumulation to 1.95 mg. of nitrogen and at 500 pounds per acre the increased accumulation was 2.60 mg. It is possible that these results cannot be taken on their face value as indirect denitrification, resulting in the liberation of ammonia could have taken place. It has not been shown however, that this organism is a denitrifier and until such data are recorded the above results would seem to be correct.

This organism is also very responsive to potash fertilization. The greatest stimulation was recorded where KCl equivalent to 300 pounds per acre was present. An approximately 20 per cent greater activity was evidenced here than where no potash was applied.

The greatest response to chemicals was exhibited where acid phosphate equivalent to 1200 pounds per acre was present. An accumulation of ammonia equivalent to 7.55 mg. of nitrogen in excess of the no treatment flasks was recorded under this treatment. In general all three appli-

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cations of acid phosphate gave way to greater activity than was induced by the addition of either nitrate of soda or chloride of potash.

TABLE XVI

THE EFFECT OF THE CHEMICAL COMPOSITION OF THE SOIL UPON THE ACTIVITY OF ZYGORHYNCHUS VUILLEMINII

Lab. No.	Soil Treatment	Ammonia Accumulation			Increase over no treatment
		Mg. N.	Mg. N.	Av. mg. N.	
341-342	100 lbs. $NaNO_3$	29.10	29.20	29.15	.70
343-344	300 lbs. $NaNO_3$	31.20	29.60	30.40	1.95
345-346	500 lbs. $NaNO_3$	30.80	31.30	31.05	2.60
347-348	100 lbs. KCl	33.90	33.90	33.90	5.45
349-350	300 lbs. KCl	34.50	34.60	34.55	6.10
351-352	500 lbs. KCl	33.90	33.30	33.60	5.15
353-354	800 lbs. Acid Phosphate	35.80	34.10	34.95	6.50
355-356	1000 lbs. Acid Phosphate	33.60	34.20	33.90	5.45
357-358	1200 lbs. Acid Phosphate	36.70	35.30	36.00	7.55
359-360	No treatment	28.00	28.90	28.45	.....

To sum up the activities of this organism under various chemical and physical conditions it seems indicative that this fungus is not very responsive to a bettered oxygen supply. This is in support of its activity in Part I under various soil types. Its response to bettered chemical environment is marked however. The greatest activity was manifested in the presence of acid phospate, the next highest in the presence of potash and the least in the presence of nitrate of soda. The chemical factor seems to be of greater importance than the physical factor.

Experiment XVII. In order to ascertain the response of *Trichoderma Koningi* to a bettered oxygen supply Experiment XVII was under-

TABLE XVII

THE EFFECT OF THE MECHANICAL COMPOSITION OF THE SOIL UPON THE ACTIVITY OF TRICHODERMA KONINGI

Lab. No.	Soil Mixture	Ammonia Accumulation			Increase over no treatment
		Mg. N.	Mg. N.	Av. mg. N.	
361-362	100 gm. loam	27.58	27.51	27.54	.....
363-364	90 gm. loam + 10 gm. SiO_2	32.69	33.25	32.97	5.43
365-366	80 gm. loam + 20 gm. SiO_2	29.75	30.59	30.17	2.63
367-368	70 gm. loam + 30 gm. SiO_2	35.07	34.34	34.70	7.16
369-370	60 gm. loam + 40 gm. SiO_2	35.63	37.73	36.63	9.09
371-372	50 gm. loam + 50 gm. SiO_2	35.98	32.55	34.26	6.72
373-374	40 gm. loam + 60 gm. SiO_2	36.23	34.30	35.26	7.72
375-376	30 gm. loam + 70 gm. SiO_2	29.05	30.80	29.92	2.38
377-378	20 gm. loam + 80 gm. SiO_2	25.76	26.34	26.05	—1.49
379-380	10 gm. loam + 90 gm. SiO_2	26.24	38.42	27.38	—.16

taken. Table XVII records the data to show the influence of a bettered oxygen supply. A casual glance at this table evidences the fact that this organism is responsive to an enhanced oxygen supply. An increase in ammonia production is to be noted on the addition of only 10 per cent of quartz sand.

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More activity was manifested with greater oxygen pressure, however, as will be seen by consulting the table. When the soil had been diluted with 70 per cent of sand a decreased activity is at hand and where 10 per cent and 20 per cent of soil are present a decreased activity in comparison with the non-diluted soil is noted.

This decrease in activity is due in no small measure to a poorer chemical environment as is evidenced by the effect of the various chemicals upon the activity of this biological factor.

Experiment XVIII. Table XVIII records the results of the influence of various chemicals upon the activity of this fungus. Upon the addition of $NaNO_3$ equivalent to 100 pounds per acre, an enhanced activity was at once recorded. An increase of 7.34 mg. is to be noted here. Larger quantities of nitrate seemed to depress the ammonifying activity of this fungus below that exhibited with the smallest amount.

TABLE XVIII

THE EFFECT OF THE CHEMICAL COMPOSITION OF THE SOIL UPON THE ACTIVITY OF TRICHODERMA KONINGI

Lab. No.	Soil Treatment	Ammonia Accumulation			Increase over no treatment
		Mg. N.	Mg. N.	Av. mg. N.	
381-382	100 lbs. $NaNO_3$	35.91	33.81	34.86	7.32
383-384	300 lbs. $NaNO_3$	33.39	29.26	31.32	3.78
385-386	500 lbs. $NaNO_3$	30.87	32.90	31.88	4.34
387-388	100 lbs. KCl	33.10	33.18	33.14	5.60
389-390	300 lbs. KCl	29.96	24.50	32.23	4.69
391-392	500 lbs. KCl	33.60	33.52	33.56	6.02
393-394	800 lbs. Acid Phosphate	30.10	27.30	28.70	1.16
395-396	1000 lbs. Acid Phosphate	29.93	29.12	29.52	1.98
397-398	1200 lbs. Acid Phosphate	27.38	30.03	28.70	1.16
399-400	No treatment	27.58	27.51	27.54	.....

Potash also stimulated activity. The increased ammonia accumulation is not so large as produced by nitrate of soda equivalent to 100 pounds per acre. No increase or depression is to be noted over the initial stimulation.

Acid phosphate in the quantities applied had very little influence upon the activity of this organism. The increased ammonia accumulation, taking into consideration all three applications, is not greater than 2 mg. of nitrogen. The data at hand, then, would seem to indicate a bettered mechanical composition of the soil as well as the presence of soluble nitrates and available potash salts are desirable for the maximum activity of this fungus.

The data in Part II have been summarized in Tables C and D, the results are tabulated as the increase or decrease of nitrogen (in milligrams) accumulated as ammonia over the portions receiving no treatment.

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This summary shows us that soil fungi may vary considerably in their oxygen requirements. Some organisms may be extremely responsive to a high oxygen pressure, whereas others may not be at all responsive to increased quantities of this element. Fungi may also have requirements that are gradient between high and low oxygen pressures.

Aspergillus niger exhibited the greatest activity when subjected to increased aeration. This fungus may be representative of those organisms which require an abundant supply of oxygen for their maximum activity.

Rhizopus tritica is representative of those organisms which show an oxygen requirement below that required for the maximum activity of the first discussed fungus.

TABLE C

SUMMARY OF THE EFFECT OF THE MECHANICAL COMPOSITION OF THE SOIL UPON THE ACTIVITY OF SOIL FUNGI

Organism	Soil alone	10% SiO_2	20% SiO_2	30% SiO_2	40% SiO_2	50% SiO_2	60% SiO_2	70% SiO_2	80% SiO_2	90% SiO_2
Aspergillus niger	.....	.23	3.09	7.32	4.67	7.06	11.30	13.90	17.55	28.77
Rhizopus tritica	.....	4.97	6.30	6.93	7.84	9.52	11.41	11.27	11.64	8.76
Zygorhynchus Vuilleminii ...	.....	1.70	—.15	1.40	0.95	.55	—3.85	2.42	—2.85	3.30
Trichoderma Koningi	.....	5.43	2.63	7.16	9.09	6.72	7.72	2.38	—1.49	—.16

Trichoderma Koningi represents a group that might get along with still less aerated conditions.

In *Zygorhynchus Vuilleminii* we have an approach to those organisms which would be very active in a very narrow atmosphere of oxygen.

The influences exerted by the various fertilizing elements have been summarized in Table D. The increase of nitrogen (in milligrams) over no treatment are reported.

The data indicate that some fungi may be quite unresponsive to chemical fertilization, whereas others may respond very well to these influences. Again the same fertilizing element may influence various fungi quite differently. For example, acid phosphate may enhance the activity of one organism (*Rhizopus tritica*), and decrease the activity of another (*Trichoderma Koningi*). In some cases no influence at all was to be noted from the amounts applied.

As was previously noted *Aspergillus niger* gave no response to any of the chemicals applied. *Zygorhynchus Vuilleminii* manifested considerable increased activity in the presence of all the fertilizing elements. *Trichoderma Koningi* responded well to small applications of nitrate of soda, but this activity was depressed by further additions. Acid phosphate caused very little increase in the activity of this fungus as measured by the accumulation of ammonia.

The greatest response to nitrate fertilization was manifested by *Trichoderma Koningi* when amounts equivalent to 100 pounds per acre of $NaNO_3$ were present. The greatest activity due to potash fertilization

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was shown by *Rhizopus tritica*. This organism also evidenced the greatest activity when acid phosphate equivalent to 800 pounds per acre were added to the soil.

A survey of both tables indicates, when one considers a specific organism, that a bettered mechanical composition may be more important in bringing about increased activity than a superior chemical environment, and *vice versa*.

TABLE D

SUMMARY OF THE EFFECT OF CHEMICAL COMPOSITION OF THE SOIL UPON THE ACTIVITY OF VARIOUS SOIL FUNGI

Organism	100 lbs. $NaNO_3$	300 lbs. $NaNO_3$	500 lbs. $NaNO_3$	100 lbs. KCl	300 lbs. KCl	500 lbs. KCl	800 lbs. A. P.	1000 lbs. A. P.	1200 lbs. A. P.
Aspergillus niger	+.70	—.50	—.20	—.05	-1.65	-1.65	—.35	.....	.....
Rhizopus tritica	6.67	6.67	6.04	6.52	6.04	6.88	7.84	6.37	6.83
Zygorhynchus Vuilleminii	.70	1.95	2.60	5.45	6.10	5.15	6.50	5.45	7.55
Trichoderma Koningi	7.35	3.78	4.34	5.60	4.69	6.02	0.98	1.98	1.16

In other instances, both an increased oxygen supply and an enhanced chemical condition are supplementary to greater activity. A more continued study of the chemical and physical factors in controlling fungus activity would seem very desirable in order to obtain more definite information in regard to the influences that these factors may regulate.

Summary of Part II

The more important points in Part II are summarized below.

Under the conditions at hand:

1. Fungi respond to an increased oxygen pressure.
2. The greatest response to oxygen supply was by *Aspergillus niger* and the least by *Zygorhynchus Vuilleminii*.
3. All the fungi studied evidenced different oxygen requirements.
4. A bettered chemical condition of the soil was followed by increased activity, with but one exception.
5. Fungi do not respond alike to the presence of fertilizing materials. The chemical favorable to one may be harmful to another.
6. To some fungi a bettered oxygen supply is of more importance than a bettered chemical supply, and vice versa.
7. Other fungi respond to both a bettered chemical and a bettered physical environment.

PART III

THE INFLUENCE OF MOISTURE UPON THE ACTIVITY OF SOIL FUNGI

Since attention to the well being of beneficial soil organisms is admittedly a vital factor in the maintainance of soil fertility, it becomes as important a question to determine the relationship of a soil moisture con-

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tent to its microbiological activities, as to ascertain the relation thereof to the growth of the plant itself. Relatively little work has been done along these lines.

Engberding (27) found that the number of bacteria increased with the water content of the soil until the latter had reached 80 per cent of saturation and that the numbers decreased when moisture was applied in larger amounts.

Lipman and Brown (24) working with a neglected clay loam soil found that ammonification increased with an increase in the moisture content even up to an amount equal to 35 per cent of the dry weight of the soil. They also found that nitrification in the same soil was most active at 15 per cent of moisture and quite perceptible at 5 per cent. Coleman (4) corroborates their results at 15 per cent, but found a marked reduction even at 10 per cent.

Lipman and Sharp (20) in a recent publication record data to show the effect of moisture on the azofication powers of a sandy loam soil. They found this biological process taking place in the presence of 4 per cent moisture and also in what they termed a virtually water-logged soil.

Nothing of any cogency has thus far come to hand which deals with the aspects of the same question with regard to the activity of soil fungi. The following data are the result of an endeavor to throw some light upon this phase of the soil biology problem. The same soil types and soil fungi as previously described in Part I were employed. In this investigation the organic matter was limited to the use of dried blood and cottonseed meal. Thus organic matter from both animal and vegetable sources was employed.

Methods

One-hundred-gm. portions of soil were weighed out, the desired organic matter added and the whole thoroughly shaken, as previously noted, and transferred to 200-c.c Erlenmeyer flasks. Tap water was added in successive portions as indicated in the tables. The flasks were sterilized as before. A preliminary experiment indicated the loss of moisture due to sterilization. By deducting this loss from the sum of the hydroscopic, added, and inoculated moisture, the percentage of actual water in the system was arrived at. It will be readily seem that conditions were created for a soil having a wide variance of moisture conditions.

Experiment XIX. Aspergillus niger having shown itself in the previous work (Part I) as an exceptionally inactive organism in the presence of dried blood, the influence of moisture on its activity was carried out with only one source of organic matter, cottonseed meal. Table XIX shows the arrangement of the experiment. Series I constitutes the sandy loam series. The uniform inoculation was, as in the previous experimentation, 1 c.c of a liquid culture of spores. The spore count was 280,000 per c.c.

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The figures seem to show that the strain of *Aspergillus niger* that we are using prefers a comparatively dry medium for its activity. The amount of ammonia accumulating decreases gradually as the moisture content increases. The remarkable fact to be noted is the consistent activity of this organism in the soil portions containing high percentages of moisture.

TABLE XIX

THE EFFECT OF MOISTURE UPON THE ACTIVITY OF ASPERGILLUS NIGER

Lab. No.	Source of Nitrogen	Per cent Moisture	Ammonia Accumulation			Inc. Mg. N.	Inoculation
			Mg. N.	Mg. N.	Av. Mg. N.		
	155 mg. N.						1 c.c. spores of
400-401	as C. S. M.	7	27.27	26.05	26.66	23.73	Aspergillus niger
402-403	"	11	23.91	24.45	24.18	21.53	"
404-405	"	13	20.07	21.00	20.53	17.86	"
406-407	"	16	19.60	20.10	19.85	17.03	"
408-409	"	18	18.92	19.35	19.13	16.26	"
410-411	"	21	14.98	14.60	14.79	12.07	"
412-413	"	23	11.72	12.03	11.87	9.20	"
414-415	"	25	13.44	14.25	13.84	10.67	"
416-417	"	28	14.50	14.90	14.70	12.03	"
418-419	"	30	16.90	13.60	15.25	11.58	"
420-421	"	33	14.02	14.00	14.01	10.47	"
422-423	"	35	4.38	4.20	4.29	0.77	"

The highest accumulation was noted with the lowest amount of moisture in the system. Beyond optimum moisture conditions, 13 per cent, a decrease in activity as measured by ammonia accumulation is to be noted. Increasing the amount of moisture beyond 21 per cent does not seem to influence the activity of this organism until a virtually saturated condition is at hand. When this point is reached the activity of this fungus at once ceases.

Experiment XX. *Penicillium* sp. 10 was used in this experiment. As was the case with *Aspergillus niger,* it is quite evident that this fungus also has its optimum, where cottonseed meal is the source of organic matter, at the lower moisture contents. With 7 per cent of moisture in the flasks the greatest activity was noted. This activity is decreased when moisture above 13 per cent is present. Further increments do not cause as depressing an effect upon the activity of this organism as was noted with *Aspergillus niger.* In comparison with this last named fungus this organism seems to desire an atmosphere somewhat more moist for its maximum activity.

Experiment XXI. As a check upon the last experiment another was performed in which the same percentage of moisture was used, but a different source of organic matter, namely dried blood. Table XXI records the data.

At the outset the activity of this fungus in the presence of this source of ammonifiable material was very low. The greatest activity seems to manifest itself along toward the saturation point of the soil. Increased

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activity is to be noted with increasing moisture conditions until the highest amount of moisture was present. In contrast to the conditions

TABLE XX

THE EFFECT OF MOISTURE UPON THE ACTIVITY OF PENICILLIUM SP. 10 WITH COTTONSEED MEAL AS THE SOURCE OF NITROGEN

Lab. No.	Source of Nitrogen	Per cent Moisture	Ammonia Accumulation			Inc. Mg. N.	Inoculation
			Mg. N.	Mg. N.	Av. Mg. N.		
425-426	155 mg. N. as C. S. M.	7	13.38	13.29	13.33	10.40	1 c.c. spores of Penicillium sp. 10
427-428	"	11	13.14	12.11	12.62	9.35	"
429-430	"	13	10.21	10.62	10.41	7.64	"
431-432	"	16	8.93	7.73	8.33	5.50	"
433-434	"	18	8.17	8.93	8.50	5.67	"
435-436	"	21	7.83	....	7.83	5.11	"
437-438	"	23	8.61	7.83	8.22	5.55	"
439-440	"	25	6.57	8.93	7.75	5.08	"
441-442	"	28	12.26	9.03	10.64	7.97	"
443-444	"	30	8.61	12.16	10.38	7.61	"
445-446	"	33	13.50	10.50	12.00	6.16	"
447-448	"	35	7.73	7.93	7.83	4.31	"

prevailing where cottonseed meal was used as the source of organic matter, a very weak activity was exhibited with small amounts of moisture in the system.

TABLE XXI

THE EFFECT OF MOISTURE UPON THE ACTIVITY OF PENICILLIUM SP. 10 WITH DRIED BLOOD AS THE SOURCE OF NITROGEN

Lab. No.	Source of Nitrogen	Per cent Moisture	Ammonia Accumulation			Inc. Mg. N.	Inoculation
			Mg. N.	Mg. N.	Av. Mg. N.		
449-450	155 mg. N. as D. B.	7	7.28	7.32	7.30	2.82	1 c.c. spores of Penicillium sp. 10
451-452	"	11	7.28	7.70	7.49	4.42	"
453-454	"	13	10.22	10.78	10.50	6.10	"
455-456	"	16	10.64	11.62	11.13	6.53	"
457-458	"	18	11.62	10.92	12.27	6.64	"
459-460	"	21	11.70	10.22	10.71	6.44	"
461-462	"	23	11.34	11.62	11.48	6.83	"
463-464	"	25	11.70	10.22	11.21	6.83	"
465-466	"	28	11.62	11.34	11.49	6.88	"
467-468	"	30	11.34	12.88	12.11	6.25	"
469-470	"	33	12.51	.....	12.51	6.11	"

Experiment XXII. Turning our attention to another group of soil organisms, the Mucoraceae, we find a different phenomenon presenting itself. Table XXII gives us some idea of the activity of *Rhizopus tritica* under varying moisture conditions. The ammonia accumulation at 0.5 optimum, 7 per cent, is nearly as large as that recorded at the highest accumulation. Increasing amounts of moisture, after the soil's optimum is reached had a very slightly depressing effect. When a nearly saturated condition is reached a marked depression is noted. On saturating the soil

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very little activity was recorded. The greatest accumulation was at optimum moisture conditions. Again, to be noted in passing is the high activity of these organisms in the presence of exceedingly high moisture conditions.

TABLE XXII

THE EFFECT OF MOISTURE UPON THE ACTIVITY OF RHIZOPUS TRITICA WITH COTTONSEED MEAL AS THE SOURCE OF NITROGEN

Lab. No.	Source of Nitrogen	Per cent Moisture	Ammonia Accumulation			Inc. Mg. N.	Inoculation
			Mg. N.	Mg. N.	Av. Mg. N.		
471-472	155 mg. N. as C. S. M.	7	34.43	34.59	34.51	31.58	1 c.c. spores of Rhizopus tritica
473-474	"	11	35.35	35.40	35.37	32.70	"
475-476	"	13	36.27	35.15	35.71	33.04	"
477-478	"	16	34.87	33.33	34.10	31.27	"
479-480	"	18	32.67	36.13	34.36	32.50	"
481-482	"	21	36.13	36.41	36.27	33.54	"
483-484	"	23	36.77	36.87	36.82	33.15	"
485-486	"	25	33.04	32.58	32.81	30.23	"
487-488	"	28	32.58	32.85	32.71	30.04	"
489-490	"	30	33.62	32.52	33.07	30.40	"
491-492	"	33	24.85	28.31	26.58	22.74	"
493-494	"	35	9.09	9.65	9.37	5.85	"

Experiment XXIII. To gain further light upon the influence of moisture on the activity of this fungus the above experiment was repeated, dried blood being substituted for cottonseed meal.

TABLE XXIII

THE EFFECT OF MOISTURE UPON THE ACTIVITY OF RHIZOPUS TRITICA

Lab. No.	Source of Nitrogen	Per cent Moisture	Ammonia Accumulation			Inc. Mg. N.	Inoculation
			Mg. N.	Mg. N.	Av. Mg. N.		
495-496	155 mg. N. as D. B.	7	18.84	17.59	18.21	13.76	1 c.c. spores of Rhizopus tritica
497-498	"	11	31.07	29.97	30.52	27.45	"
499-500	"	13	31.45	31.45	31.45	27.05	"
501-502	"	16	29.25	32.33	30.79	26.19	"
503-504	"	18	30.93	32.47	31.70	27.77	"
505-506	"	21	31.07	31.07	31.07	26.80	"
507-508	"	23	30.93	29.10	30.03	25.45	"
509-510	"	25	28.88	30.93	29.90	25.60	"
511-512	"	28	30.93	30.65	30.79	26.18	"
513-514	"	30	4.97	5.22	5.08	2.22	"
515-516	"	33	4.38	4.58	4.46	1.06	"

Very little activity is to be noted in the presence of dried blood with low amounts of moisture in the soil contrasting with the results where cottonseed meal was used. Increasing the percentage of moisture however, results in increased activity. When optimum moisture conditions prevail the highest activity is to be recorded. When additional moisture is present a depression in the activity of this organism commences to

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manifest itself. High activity is at hand, however, with large percentages of moisture in the system. When a saturated condition is reached the activity of the fungus is exceedingly weak, and when a water-logged condition is reached, very little, if any, activity is to be noted.

In this experiment the greatest activity is again at the soil's optimum moisture conditions. This experiment checks the preceding one very well in this respect. The only striking difference to be noted is that dried blood is ammonified to a less extent by this fungus than cottonseed meal in soil having moisture contents near one-half optimum. This lower activity, in the presence of dried blood with low moisture contents, of both *Rhizopus tritica* and *Penicillium* sp. seems to suggest that more favorable moisture conditions are necessary for the activity of these organisms where dried blood is used as a source of ammonifiable material. On the other hand, cottonseed meal seems to promote high activity even in soil with an exceedingly low moisture content.

Experiment XXIV. Giving our attention to one of the other members of this group, *Zygorhynchus Vuilleminii* we note a very similar arrangement of results. Table XXIV records the data showing the influ-

TABLE XXIV

THE EFFECT OF MOISTURE UPON THE ACTIVITY OF ZYGORHYNCHUS VUILLEMINII WITH COTTONSEED MEAL AS THE SOURCE OF NITROGEN

Lab. No.	Source of Nitrogen	Per cent Moisture	Ammonia Accumulation			Inc. Mg. N.	Inoculation
			Mg. N.	Mg. N.	Av. Mg. N.		
517-518	155 mg. N. as C. S. M.	7	29.40	28.35	28.87	25.94	1 c.c. spores of Zygorhynchus Vuilleminii[1]
519-520	"	11	32.40	31.80	32.10	29.43	"
521-522	"	13	31.63	32.08	31.85	28.18	"
523-524	"	16	32.94	32.19	32.56	29.73	"
525-526	"	18	33.40	32.17	32.78	29.91	"
527-528	"	21	31.40	31.40	31.40	28.68	"
529-530	"	23	29.54	32.17	30.85	28.18	"
531-532	"	25	32.45	30.17	31.31	28.64	"
533-534	"	28	32.17	31.72	31.94	29.27	"
535-536	"	30	31.87	31.32	31.59	28.92	"
537-538	"	33	29.79	29.79	29.79	25.95	"
539-540	"	35	9.41	9.25	9.33	5.81	"

[1] 58,000 spores per c.c.

ence of moisture upon the activity of this fungus, when cottonseed meal is used as a source of organic matter. The results seem to be in accord with those secured in the experimentation with *Rhizopus tritica*. With 7 per cent of moisture in the soil we have exactly the same accumulation of ammonia as with 33 per cent or a nearly saturated condition, truly a remarkable adaptation to moisture conditions. On increasing the amount of moisture from one-half optimum to optimum, we have a gradual increase in the activity of this fungus. This activity remains practically

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constant with increasing moisture contents until an approximately saturated condition is reached. There is then, upon increased moisture conditions a rapid and pronounced depression in the ammonifying power of this fungus. There seems to be no exact point giving the highest ammonia accumulation. Any point between 11 per cent and 30 per cent seems to be just as favorable as the best moisture conditions for the ammonifying efficiency of this organism.

Experiment XXV. A duplication of the above experiment with dried blood in place of cottonseed meal gives similar results. Table XXV records the data. It is again seen that this fungus is very tolerant of high moisture conditions. At one-half optimum, 2.55 mg. of nitrogen accumu-

TABLE XXV

THE EFFECT OF MOISTURE UPON THE ACTIVITY OF ZYGORHYNCHUS VUILLEMINII WITH DRIED BLOOD AS THE SOURCE OF NITROGEN

Lab. No.	Source of Nitrogen	Per cent Moisture	Ammonia Accumulation			Mg. N. Inc.	Inoculation
			Mg. N.	Mg. N.	Mg. N. Av.		
	155 mg. N.						1 c.c. spores of
541-542	as D. B.	7	6.95	7.05	7.00	2.55	Zygorhynchus Vuilleminii[1]
543-544	"	11	8.64	8.50	8.57	5.50	"
545-546	"	13	10.00	10.00	10.00	5.60	"
547-548	"	16	8.79	8.65	8.72	4.12	"
549-550	"	18	7.87	7.87	7.87	3.24	"
551-552	"	21	8.64	8.50	8.57	4.30	"
553-554	"	23	8.90	8.52	8.71	4.13	"
555-556	"	25	9.10	8.64	8.87	4.47	"
557-558	"	28	9.72	9.30	9.51	4.90	"
559-560	"	30	4.79	4.79	4.79	.93	"
561-562	"	33	4.49	4.79	4.49	1.09	"

[1] 58,000 spores per c.c.

lated, and at optimum 5.60 mg. Further additions cause a slight decrease in the ammonifying efficiency of this organism. The accumulation at optimum was nearly as great as it was with 28 per cent of moisture in the soil. The same conditions which concerned the activity of the previous fungi in the presence of dried blood in soils of small moisture contents are at hand here.

Experiment XXVI. The following work was carried out with *Trichoderma Koningi.* An examination of Table XXVI shows that this fungus is about as active in soils of extremely low and high water contents. We received here the largest ammonification of cottonseed meal at extremely low moisture contents of any of the fungi studied. We also received at one-half optimum as much as at saturation. In fact, greater mycelial growth was noted with the higher moisture contents. In contrast to the other fungi studied, this organism is very active in a virtually water-logged soil, 34.56 mg. of nitrogen having accumulated out of the possible 155 mg. at this moisture content!

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TABLE XXVI

THE EFFECT OF MOISTURE UPON THE ACTIVITY OF TRICHODERMA KONINGI WITH COTTONSEED MEAL AS THE SOURCE OF NITROGEN

Lab. No.	Source of Nitrogen	Per cent Moisture	Ammonia Accumulation			Mg. N. Inc.	Inoculation
			Mg. N.	Mg. N.	Mg. N. Av.		
563-564	155 mg. N. as C. S. M.	7	35.94	42.81	39.37	36.44	1 c.c. spores of Trichoderma Koningi[1]
565-566	"	11	49.44	49.40	49.92	46.75	"
567-568	"	13	50.44	50.40	50.42	47.75	"
569-570	"	16	49.41	49.56	49.48	46.63	"
571-572	"	18	50.44	50.40	50.42	47.55	"
573-574	"	21	50.44	49.99	50.21	47.49	"
575-576	"	23	47.37	46.10	46.73	44.06	"
577-578	"	25	49.91	49.16	49.53	46.86	"
579-580	"	28	52.81	56.36	51.58	48.91	"
581-582	"	30	42.67	45.97	44.32	41.65	"
583-584	"	33	41.43	41.88	41.65	37.81	"
585-586	"	35	40.44	35.75	38.08	34.56	"

[1] 278,000 spores per c.c.

Experiment XXVII. As was the case in the previous experiments the above experiment was repeated with dried blood as the source of organic matter.

TABLE XXVII

THE EFFECT OF MOISTURE UPON THE ACTIVITY OF TRICHODERMA KONINGI WITH DRIED BLOOD AS THE SOURCE OF NITROGEN

No. Lab.	Nitrogen Source of	Moisture Per cent	Ammonia Accumulation			Inc. Mg. N.	Inoculation
			Mg. N.	Mg. N.	Av. Mg. N.		
587-588	155 mg. N. as D. B.	7	29.91	30.22	30.06	27.32	1 c.c. spores of Trichoderma Koningi[1]
589-590	"	11	37.91	37.17	37.54	34.80	"
591-592	"	13	37.30	39.37	38.33	35.59	"
593-594	"	16	41.30	39.76	40.53	37.79	"
595-596	"	18	45.72	44.55	45.13	42.39	"
597-598	"	21	44.41	50.74	47.57	44.83	"
599-600	"	23	49.19	60.79	54.99	52.25	"
601-602	"	25	57.98	61.83	59.90	57.16	"
603-604	"	28	56.04	60.75	58.39	55.65	"
605-606	"	30	47.32	50.06	48.69	45.95	"
607-608	"	33	10.53	6.81	8.67	6.13	"

[1] 278,000 spores per c.c.

Less ammonification took place at lower moisture contents with dried blood as the source of organic matter than where cottonseed meal was employed. The increase in ammonia formation is more pronounced with this material. The highest ammonification took place with 25 per cent moisture in the system. Although ammonia was found at saturated conditions, its accumulation was not as strong as was the case with cotton-

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seed meal. The optimum moisture content for this soil, 13 per cent, does not promote the greatest activity of this organism. Conditions more closly approximate to one and one-half optimum give the highest accumulation of ammonia.

Discussion

The data from Series I have been summarized in Tables E and F. An examination of these tables shows one some interesting facts about the activity of soil fungi as influenced by varying moisture conditions.

TABLE E

ACCUMULATION OF NITROGEN (MG.) FROM THE AMMONIFICATION OF COTTONSEED MEAL

Name of Organism	Percentage Moisture											
	7	11	13	16	18	21	23	25	28	30	33	35
Aspergillus niger	23.73	21.53	17.86	17.03	16.26	12.07	9.26	10.67	12.03	11.58	10.47	.77
Penicillium sp. 10	10.40	9.35	7.64	5.50	5.65	5.11	5.55	5.08	7.97	7.61	6.16	4.31
Rhizopus tritica	31.58	32.70	33.04	31.27	32.50	33.54	33.15	30.23	30.04	30.40	22.74	5.85
Zygorhynchus Vuilleminii ...	25.94	29.43	28.18	29.73	29.91	28.68	28.18	28.64	29.27	28.92	25.95	5.81
Trichoderma Koningi	36.41	46.75	47.75	46.63	47.55	47.49	44.06	46.86	48.91	41.65	37.81	34.56

There seems to be no critical point which is favorable to the maximum activity of all five forms studied. The two members of the Aspergillaceae, on the whole, seem to require a somewhat drier medium for their activities than do the other forms studied.

TABLE F

ACCUMULATION OF NITROGEN (MG.) FROM THE AMMONIFICATION OF DRIED BLOOD

Name of Organism	Percentage of Moisture										
	7	11	13	16	18	21	23	25	28	30	33
Aspergillus niger	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium sp. 10	2.82	4.42	6.10	6.53	6.64	6.44	6.83	6.83	1.88	6.25	6.11
Rhizopus tritica	13.76	27.45	27.05	26.19	27.77	26.80	25.45	25.60	26.14	2.22	1.06
Zygorhynchus Vuilleminii	2.55	5.50	5.60	4.12	3.24	4.30	4.13	4.47	4.90	.93	1.03
Trichoderma Koningi...	27.32	34.20	35.59	37.79	42.39	44.83	52.25	57.16	55.65	45.95	6.13

Aspergillus niger required the driest medium for its maximum activity. The species of *Penicillium* enjoyed one somewhat more moist.

Rhizopus tritica seemed to have its greatest activity at optimum moisture conditions in the presence of both sources of organic matter.

Zygorhynchus Vuilleminii requires a least optimum moisture conditions for its greatest activity. Given this condition, increased moisture has but little influence upon its activities until the oxygen supply is entirely cut off.

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Trichoderma Koningi has a truly remarkable adaptation to moisture conditions. This organism was very active at one-half optimum and at saturated conditions. Optimum conditions for the activity of this fungus seem to be at hand when moisture conditions are present equal to one and one-half the soil's optimum.

The accumulated data suggest that soil fungi have a wide variance as to moisture requirements. Some fungi may require a dry medium for their maximum activity as is illustrated by the members of the Aspergillaceae studied. Other fungi, however, may have their maximum activity when the soil's moisture content is at optimum (*Rhizopus tritica*). Still other organisms might be more active under high moisture conditions. *Trichoderma Koningi* is illustrative of this point.

The high activity of these organisms in the presence of excessive moisture might also aid in explaining the fertility of many soils which are endowed with a high water content during a greater portion of the year. Necessarily, in these soils, bacterial activities would be profoundly affected. If the fungi, however, are tolerant of these high moisture conditions decomposition processes would still continue. This phenomenon is illustrated in cranberry bogs which are covered with water a great portion of the year. Many of these are high in fetility and do not respond to fertilizer treatment. May not the fungi be instrumental in causing the high fertility of these soils?

Of peculiar interest are the phenomena previously mentioned relative to the moisture requirements for the activity of fungi in the presence of dried blood, i. e., the smaller ammonification of dried blood at lower moisture conditions in comparison with cottonseed meal under similar moisture conditions. Why this obtains the writer is unable to say. It may be due to some enzymatic action that does not take place under dry conditions.

The cessation of activities when saturated conditions were reached was due no doubt to a cutting off of the oxygen supply.

Summary of Part III

Results are given which show:

1 Wide differences exist in the moisture requirements of soil fungi.

2. *Aspergillus niger* is favored by a very dry medium for its maximum activity.

3. *Rhizopus tritica* is most active at optimum moisture conditions.

4. *Trichoderma Koningi* is favored by a moist medium.

5. *Zygorhynchus Vuilleminii* has a wide moisture adaptability.

6. A greater amount of moisture is necessary to ammonify dried blood than cottonseed meal.

7. Five fungi were studied, two sources of organic matter being used and moisture conditions varying from 7 to 35 per cent.

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PART IV

THE INFLUENCE OF TEMPERATURE UPON THE ACTIVITY OF SOIL FUNGI

Temperature as well as moisture is one of the more important factors influencing the activity of soil organisms. Seasonal variations in temperature are well known facts. It is obvious therefore, because of these different temperature variations microbial life must be profoundly modified. The optimum temperature which allows the most active growth is quite different for various species.

The majority of organisms, in fact all living organisms, have their optimum temperature between 20° and 40° C. The organisms found in the soil are of various natures. In the summer, soil under the radiation of the sun often reaches high temperatures. A greater number of the organisms will therefore, have their optimum nearer 40° C. than 30° C.

The literature on temperature as a factor influencing biological activities is quite extensive. A detailed resumé is not in place here. In passing it is noteworthy to mention that Conn (5) finds higher numbers of bacteria in the winter than in the summer. Möller (29) observed a slight evolution of carbon dioxide from frozen soils and Wollny (41) has data to show that a rise in the soil's temperature is accompanied by an increased production of carbon dioxide. It is quite evident then, that biological activities are not suspended at relatively low temperatures and are enhanced by higher temperatures. Considering specific groups of soil bacteria, one finds that nitrification is feeble at 5° C., perceptible at 12° C., and optimum at 37° C. At 15° C. the breaking down of organic matter is fairly rapid and at 25° C. we find the optimum for many species. Ammonification has been shown by Marchal (27) to be extremely feeble at 5° C. At 20° C. ammonia production was marked and at 30° C. the maximum production was reached.

In a recent publication Traaen (38) has endeavored to study the temperature requirements of some of the soil fungi which he isolated. His method was to measure the radical growth of the organism when grown under various temperatures upon agar plates. The only organism related to those studied here was *Trichoderma Vignorium* which had its optimum temperature at about 18° to 20° C.

No other data having come to hand bearing upon this question it becomes of interest to note just what effect different temperatures would have on the activity of soil fungi. Consequently a series of experiments was outlined to test the activity of the fungi previously studied under various conditions of temperature. The thermal points chosen were 6° to 8° C., 15° to 17° C., 22° to 25° C., 30° C. and 38° C.

Cottonseed meal, having been shown to be an excellent source of organic matter for the activity of soil fungi, was used as the ammonifiable material. The same soils were used as previously described. The fungi

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used were those named in Part I. Methods of procedure, analyses, etc., were identical with the previous work.

Series I

Experiment XXVIII. ***Aspergillus niger*** was the first organism studied. A survey of Table XXVIII will show the reader the wonderful response that this fungus exhibits to high temperature conditions. At 6° to 8° C. no ammonification was detected. Very little

TABLE XXVIII

THE INFLUENCE OF TEMPERATURE UPON THE ACTIVITY OF ASPERGILLUS NIGER IN SANDY SOIL

Lab. No.	Temp. ° C.	Ammonia Accumulation			Increase	Inoculation
		Mg. N.	Mg. N.	Av.Mg.N.		
711-712	6-8	3.30	2.91	3.10	....	1 c.c. spores of Aspergillus niger
713-714	15-17	3.34	2.96	3.15	....	"
715-716	22-25	11.10	9.70	10.40	7.30	"
717-718	30	57.00	58.90	57.95	54.85	"
719-720	38	75.90	78.30	77.10	74.00	"

growth was present in the flask. It was barely noticeable microscopically. At 15° to 17° C., growth was present. The organism was very inactive, however, as no accumulated ammonia could be detected. A slightly enhanced activity was manifested at 22° C. Where the temperature was elevated to 30° C. the fungus became exceedingly active. The amount of ammonia accumulating being nearly 8 times as great as was present at room temperature or 22° C. On raising the temperature to 38° C. still greater activity was to be noted, 74 mg. of nitrogen having accumulated at the end of the incubation period. The optimum temperature for this organism is very high. Perhaps even beyond 38° C. as no diminution of activity was recorded in comparison with 30° C. Marshall (28) is authority for the statement that some strains of this organism enjoy a temperature of 44° C. Also to be noted, is the fact that this organism exhibited no activity, indeed no visible growth at the lowest temperature employed. No decomposition was recorded even at the fairly high temperature of 15° to 17° C. Growth, however, was present. The minimum temperature, therefore, for the activity of this organism must be close to 15° C., and the maximum beyond 38° C. This marked preference for high temperatures explains very well the weak activity of this organism at the lower temperatures.

Experiment XXIX. Table XXIX records the data showing the influence of temperature upon the activity of the species of Penicillium at hand.

At the lowest temperature employed this fungus was very inactive as measured by the ammonia accumulated. Growth, however, was present.

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It was very meager. No doubt all the ammonia that might have formed was used by the organism for its life process. The greatest activity was noted at 30° C., 31.78 mg. of ammonia having accumulated at this point. Increase in temperature to 38° C. apparently stopped the ammonifying power of this fungus. The optimum temperature for the activity of this organism appears to be between 30° and 38° C. The activity of the organism is approximately 5 times as great at 30° C. as it is at 22° C.

TABLE XXIX

THE INFLUENCE OF TEMPERATURE UPON THE ACTIVITY OF PENICILLIUM SP. 10 IN SANDY SOIL

Lab. No.	Temp. ° C.	Ammonia Accumulation			Increase	Inoculation
		Mg. N.	Mg. N.	Av.Mg.N.		
721-722	6-8	3.34	2.91	3.12	....	1 c.c. spores of Penicillium sp. 10
723-724	15-17	9.59	8.17	8.88	5.41	"
725-726	22-25	10.59	9.59	10.09	6.62	"
727-728	30	31.78	30.60	31.19	28.07	"
729-730	38	3.66	.....	3.66	.....	"

Experiment XXX. Considering the two members of the Mucoraceae we find in both cases a different status of affairs. Table XXX records the effect of temperature upon the ammonifying efficiency of *Rhizopus tritica.*

TABLE XXX

THE INFLUENCE OF TEMPERATURE UPON THE ACTIVITY OF RHIZOPUS TRITICA IN SANDY SOIL

Lab. No.	Temp. ° C.	Ammonia Accumulation			Increase	Inoculation
		Mg. N.	Mg. N.	Av.Mg.N.		
731-732	6-8	4.13	4.27	4.20	0.85	1 c.c. spores of Rhizopus tritica
733-734	15-17	14.66	14.51	14.58	11.13	"
735-736	22-25	37.95	38.70	38.32	34.87	"
737-738	30	48.54	48.09	48.30	44.85	"
739-740	38	19.69	24.71	22.20	18.75	"

As was noted in the experiment with the preceeding fungus, no ammonification took place at 6° to 8° C. No growth manifested itself at this temperature. At 15° to 17° C. considerable ammonia accumulation is obtained. This accumulation is proportional to the increase in temperature until a point between 30° and 38° C. is reached. At 38° C. the accumulation is greater than at 15° C., but is less than at 22° or 30° C. It is quite evident that this organism's activity is favored by rather high temperatures. The optimum temperature seems to be beyond 30° C. and the maximum is reached beyond 38° C. The minimum temperature seems to be between 8° and 15° C. This fungus is a fairly active organism at comparatively low temperatures. It is of interest to note in passing that

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Ames (1) has found that 36° C. is the optimum temperature for the Rhizopus causing the storage rot of sweet potatoes.

Experiment XXXI. The activities of *Zygorhynchus Vuilleminii* are in a measure similar to those of the other members of this group. Table XXXI gives the data showing the influence of temperature upon the activity of this fungus. No ammonia or growth was detected at 6° to 8° C. The activity manifested at 15° to 17° C. was the greatest for all of the

TABLE XXXI

THE INFLUENCE OF TEMPERATURE UPON THE ACTIVITY OF ZYGORHYNCHUS VUILLEMINII IN SANDY SOIL

Lab. No.	Temp. ° C.	Ammonia Accumulation			Increase	Inoculation
		Mg. N.	Mg. N.	Av.Mg.N.		
741-742	6-8	2.89	3.19	3.04	.41	1 c.c. spores of Zygorhynchus Vuilleminii
743-744	15-17	22.73	21.83	22.28	19.63	"
745-746	22-25	29.30	29.15	29.22	25.77	"
747-748	30	56.84	57.04	56.94	53.84	"
749-750	38	4.56	4.56	4.56	1.11	"

fungi studied, at this temperature 19.63 mg. of ammonia having accumulated at this temperature. The largest accumulation was at 30° C. No activity was recorded at 38° C. This fungus seems to be the most active one at hand at a temperature of 15° to 17° C. The optimum is probably between 30° and 38° C. and the minimum between 8° and 15° C.

Experiment XXXII. Taking up one of the other groups of organisms we note that it also, is favored by high temperature. *Trichoderma Koningi* was the organism studied. At 6° to 8° C. no ammonia accumulated. At 15° C. an accumulation of 9.62 mg. of nitrogen was noted.

TABLE XXXII

THE INFLUENCE OF TEMPERATURE UPON THE ACTIVITY OF TRICHODERMA KONINGI IN SANDY SOIL

Lab. No.	Temp. ° C.	Ammonia Accumulation			Increase	Inoculation
		Mg. N.	Mg. N.	Av.Mg.N.		
751-752	6-8	2.48	4.90	3.69	.22	1 c.c. spores of Trichoderma Koningi
753-754	15-17	2.44	13.75	13.09	9.62	"
755-756	22-25	31.21	39.31	37.83	34.38	"
757-758	30	36.40	29.80	30.50	27.03	"
759-760	38	15.00	.....	15.00	11.53	"

This is second in rank to the activity shown by *Zygorhynchus Vuilleminii* at this temperature. The greatest activity was experienced at 30° C. Upon raising the temperature to 38° C., the ammonia accumulation ceased indicating that the maximum thermal point had been reached. In this soil type the optimum temperature for this fungus' activity is in the vicinity of 30° C. The minimum temperature seems to be between 8° and 15° C.

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A resumé of the influence of temperature upon the activity of the organisms in this series is graphically represented in figure 3.

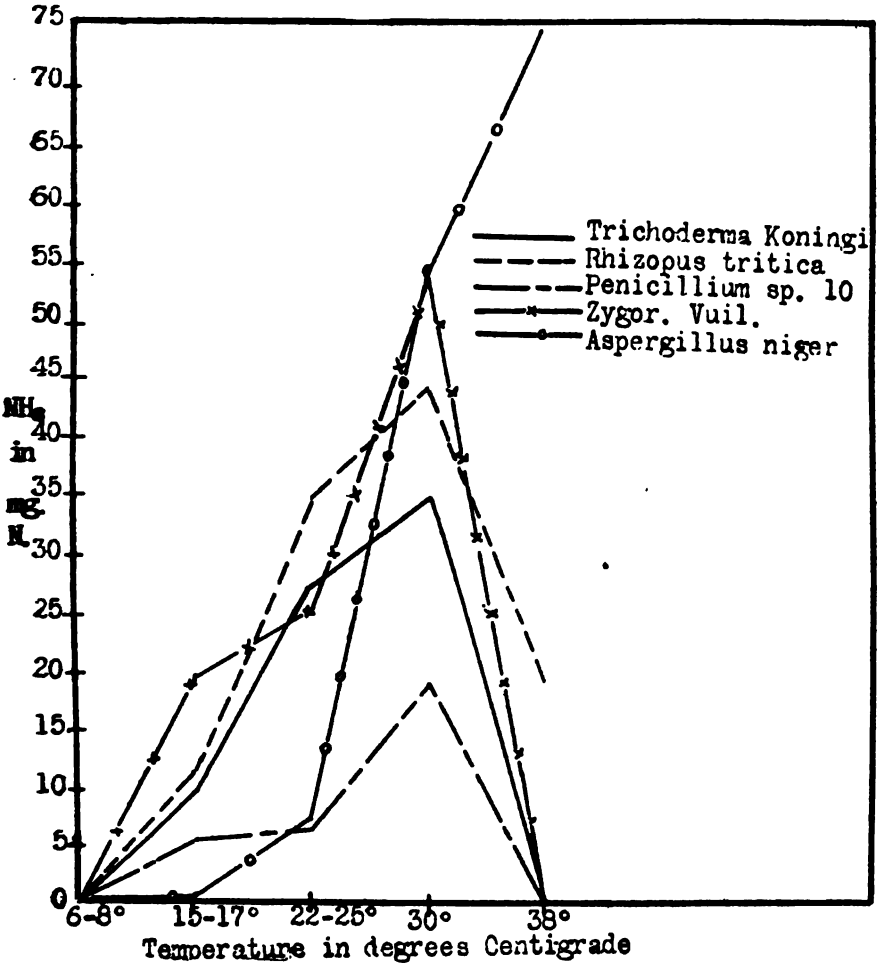

Fig. 3.—Diagram showing the influence of temperature on the activities of soil fungi.

Series II

As a check upon the above series and to give some additional information in regard to the activity of soil fungi under different temperature conditions a second series was executed, in which a heavy clay loam was used in place of the sandy loam previously used. This series is in every respect identical with Series I. Both series received the same inoculation and were incubated at the same time so that all factors are strictly comparable.

Experiment XXXIII. The results reported in Series I, Experiment XXVII are duplicated in every way in this experiment. No activity is

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to be detected by ammonia accumulation either at 6° to 8° C. or 15° C., and even at 22° C. very little activity is to be seen. Again, however, on raising the temperature to 30° C., an increased activity is recorded. The response is in about the same proportion as previously stated, perhaps a bit greater. No mycelial growth was present at the lowest temperature in this type of soil. At 15° C. a scant mycelial growth was noted with but few spores present. At 22° C. sporulation had taken place. The 30° C. cultures had a dense black, oily mass of mycelium and spores covering the surface of the soil. When a temperature of 38° C. was reached the greatest activity was again noted, 59.30 mg. of nitrogen being found at the end of 7 days. This is in confirmation of the work in Series 1.

TABLE XXXIII
THE INFLUENCE OF TEMPERATURE UPON THE ACTIVITY OF ASPERGILLUS NIGER IN CLAY SOIL

Lab. No.	Temp. ° C.	Ammonia Accumulation			Increase	Inoculation
		Mg. N.	Mg. N.	Av.Mg.N.		
761-762	6-8	3.80	3.60	3.60	....	1 c.c. spores of Aspergillus niger
763-764	15-17	3.80	3.70	3.75	....	"
765-766	22-25	8.80	9.30	9.05	5.50	"
767-768	30	49.90	51.10	50.50	46.90	"
769-770	38	62.27	63.58	62.92	59.30	"

Experiment XXXIV. The data recorded in Table XXXIV give the influence of temperature upon the activity of *Penicillium* sp. 10 in this series. In general the data are in confirmation of Series I. No accumulation of ammonia was noted at 6° to 8° C. A little more than half as much ammonia was accumulated at 15° C. as was accumulated at 22° C.

TABLE XXXIV
THE INFLUENCE OF TEMPERATURE UPON THE ACTIVITY OF PENICILLIUM SP. 10 IN CLAY SOIL

Lab. No.	Temp. ° C.	Ammonia Accumulation			Increase	Inoculation
		Mg. N.	Mg. N.	Av.Mg.N.		
771-772	6-8	4.47	6.04	5.25	—.28	1 c.c. spores of Penicillium sp. 10
773-774	15-17	7.46	7.46	7.46	1.83	"
775-776	22-25	7.82	9.88	8.85	3.22	"
777-778	30	23.94	23.16	23.55	18.79	"
779-780	38	4.76	4.76	4.76	—.80	"

The point is again brought out that this fungus has its optimum thermal point at about 30° C. No accumulation of ammonia, or growth of the organism was noted at 38° C. Growth however, was present at 6° to 8° C. although no ammonia accumulated.

Experiment XXXV. The same phenomena hold true with Mucoraceae in this series as was evidenced in Series I. Table XXXV records the data developed from the experimentation with *Rhizopus tritica.* At 6° to 8° C. no growth was noted. A very little ammonia accumulated at

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15° to 17° C. Growth of the organism was also very weak. The highest accumulation was obtained at 30° C. An advance in temperature to 38° C. causes a decrease in the activity of this fungus as is evidenced by the ammonia present at the end of the incubation period. A change in

TABLE XXXV

THE INFLUENCE OF TEMPERATURE UPON THE ACTIVITY OF RHIZOPUS TRITICA IN CLAY SOIL

Lab. No.	Temp. °C.	Ammonia Accumulation			Increase	Inoculation
		Mg. N.	Mg. N.	Av.Mg.N.		
781-790	6-8	4.91	4.76	4.83	2.98	1 c.c. spores of Rhizopus tritica
791-792	15-17	7.91	8.21	8.06	2.25	"
793-794	22-25	16.57	18.52	17.54	11.73	"
795-796	30	32.92	31.87	32.39	26.58	"
797-798	38	29.73	29.71	29.72	23.93	"

the soil type causes a change in minimum temperature for this organism's activity. In this series it is nearer 15° C. The optimum temperature is nearer 38° C. Necessarily the maximum would be higher in this series.

Experiment XXXVI. Considering next the activity of *Zygorhynchus Vuilleminii* in this series, we again have evidence to show that this fungus is an efficient ammonifier at fairly low temperatures. Table XXXVI records the data.

TABLE XXXVI

THE INFLUENCE OF TEMPERATURE UPON THE ACTIVITY OF ZYGORHYNCHUS VUILLEMINII IN CLAY SOIL

Lab. No.	Temp. °C.	Ammonia Accumulation			Increase	Inoculation
		Mg. N.	Mg. N.	Av.Mg.N.		
791-792	6-8	4.44	3.94	4.19	—.73	1 c.c. spores of Zygorhynchus Vuilleminii
793-794	15-17	12.74	12.62	12.68	6.76	"
795-796	22-25	21.01	21.84	21.42	15.50	"
797-798	30	34.79	33.65	34.22	29.30	"
799-800	38	4.46	4.84	4.65	—1.06	"

No ammonia accumulated at 6° to 8° C. At 15° to 17° C., 6.72 mg. was found and at room temperature, 22° C. the next highest activity was exhibited. The highest accumulation of all was found at 30° C. A temperature of 38° C. was inimical to the activity of this fungus. The optimum temperature again seems to be within 30° to 38° C., and the minimum between 8° and 15° C. This experiment is in confirmation of the work in Series I.

Experiment XXXVII. Trichoderma Koningi also exhibits the same order of activity in this series. Table XXXVII tabulates the data. Here again 30° C. is the optimum temperature. No activity was noticed at

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38° C. More activity was noted at 15° C. with this organism than was exhibited by any of the other organisms at this temperature.

TABLE XXXVII

THE INFLUENCE OF TEMPERATURE UPON THE ACTIVITY OF TRICHODERMA KONINGI IN CLAY SOIL

Lab. No.	Temp. °C.	Ammonia Accumulation			Increase	Inoculation
		Mg. N.	Mg. N.	Av.Mg.N.		
801-802	6-8	4.46	4.49	4.47	—1.72	1 c.c. spores of Trichoderma Koningi
803-804	15-17	13.21	13.63	13.42	7.02	"
805-806	22-25	36.59	35.40	35.99	33.50	"
807-808	30	52.83	51.90	52.36	49.24	"
809-810	38	4.65	4.53	4.59	—.13	"

In order to bring out the effect of temperature on the activity of the fungi studied the data from both series have been summarized in Table G and the results of Series II are graphically represented in figure 4.

TABLE G

SUMMARY TABLE SHOWING THE INFLUENCE OF TEMPERATURE UPON THE ACTIVITY OF SOIL FUNGI

Name of Organism	Ammonia accumulated (mg. N.) Sandy Soil					Ammonia accumulated (mg. N.) Clay Soil				
	6°-8°	15°-17°	22°-25°	30°	38°	6°-8°	15°-17°	22°-25°	30°	38°
Aspergillus niger	.....	.....	7.30	54.85	74.00	.....	.....	5.50	46.90	59.30
Penicillium sp. 10	.....	5.41	6.62	28.07	.....	—.28	1.83	3.22	18.79	—.80
Rhizopus tritica	.85	11.13	34.87	44.85	18.75	—.98	2.25	11.73	26.58	23.93
Zygorrhynchus Vuilleminii ..	.41	19.63	25.77	53.84	.....	—.73	6.76	15.50	29.30	-1.06
Trichoderma Koningi	.22	9.62	27.03	34.38	11.53?	-1.72	7.02	33.50	49.24	-1.30

Discussion

The data appended in Table G indicate a rather narrow temperature range for the activity of soil fungi. No activity as evidenced by ammonia accumulation, was noted in all ten trials at 6° to 8° C. In some cases a loss is noted which may be attributed to consumption of any accumulated ammonia.

At 15° to 17° C., *Penicillium* sp. 10, *Trichoderma Koningi* and *Zygorhynchus Vuilleminii* all showed marked activity. The optimum temperature for the majority of the fungi studied was around 30° to 35° C. It is the opinion of the writer that this temperature should be chosen for studies with soil fungi as the maximum activity of the forms studied was noted here.

Considering some specific instances, *Rhizopus tritica* was very active at 38° C., whereas all the other organisms with the exception of *Aspergillus niger* were inactive at this degree of heat.

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At 30° C. *Aspergillus niger* was the most active organism.

The greatest activity at 15° to 17° C., was shown by *Zygorhynchus Vuilleminii*, the next greatest activity by *Trichoderma Koningi*.

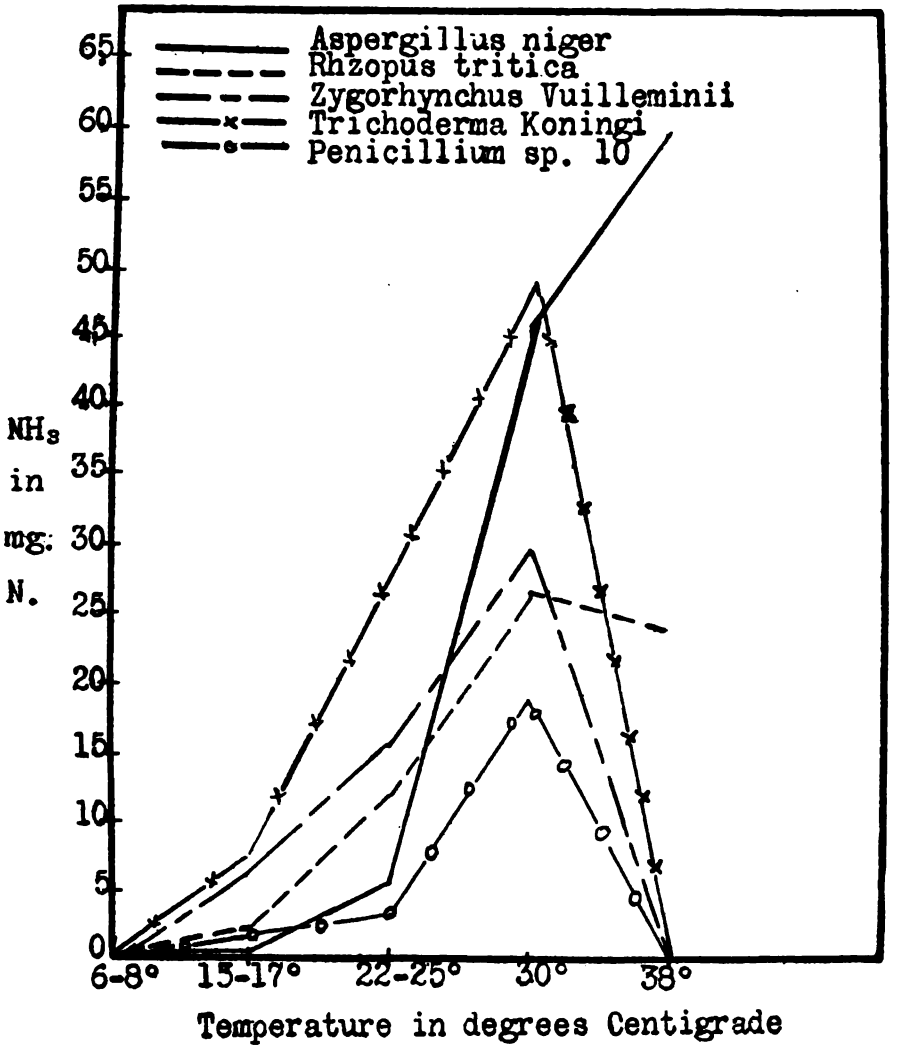

Fig. 4.—Diagram showing the influence of temperature on the activities of soil fungi.

Summary of Part IV

To summarize the work in Part IV the following is tabulated:

1. A 7-day incubation period, one source of organic matter and two types of soil were used as standards.

2. Five species of fungi were experimented upon.

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3. Five temperatures were employed; 6° to 8° C., 15° to 17° C., 22° C., 30° C. and 38° C.

4. The data indicate that soil fungi have a narrow temperature range.

5. No activity was recorded in any case at 6° to 8° C. as measured by ammonia accumulation. Growth however was noted in several instances, with a loss of accumulated ammonia.

6. The greatest activity at a temperature of 15° to 17° C. was exhibited by *Zygorhynchus Vuilleminii.*

8. The highest accumulation at a temperature of 22° to 25° C. was shown by *Rhizopus tritica* in both types of soil.

9. At 30° C. *Aspergillus niger* was the most active organism.

10. The minimum temperature for the activity of soil fungi seems to be within 8° and 15° C.

11. The maximum for most of the species at hand was between 30° and 38° C.

12. The optimum temperature for the study of soil fungi should be 30° C. rather than lower temperatures.

13. The soil may be a determining factor in influencing the heat relations of soil fungi.

14. *Aspergillus niger* was the only organism to show no activity at 15° to 17° C.

15. *Aspergillus niger* was also the most active at 38° C.

Part V

The Associative Action of Soil Fungi and Soil Bacteria

A never failing factor, bound to influence the activity of soil fungi, is their companion organisms in the soil, the bacteria. These two groups of organisms are always together in all types of soil. Indeed it would be a great rarity to obtain a sample of soil with a pure culture of either group of organisms.

Soils are variable in their character of composition. Some soils are very sandy, or open, others are close and heavy. The amounts and proportions of the mineral elements vary to a considerable extent with the various types of soil. In a similar manner the organic matter in the various soil types is of a varied nature, both as to quality and quantity. When one also considers in addition to the above the wide diversity of soil microörganisms with regard to their attitude to the above mentioned conditions, we can at once conceive of many ways in which existing microbial equilibriums, of which soil fungi and soil bacteria are highly representative, could be variously altered. For example, it can be conceived that the addition of dried blood to a soil, in which the predominating flora is of a fungus character, could so favor the growth of bacteria that the group relationship would be very materially changed, and *vice versa,* if cottonseed meal should be used where the flora is highly bacterial in

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nature. One can also conceive of alteration in group relations by a change in soil type, a more open soil affording the development of some species to the depression of others. Within the same soil type various seasonal factors may have an important influence upon various soil groups. The amount and distribution of rainfall within a given season, and even within the same soil mass (5) is bound to effect changes in a manner of which we may never have any conception. In a like manner the variations in temperature may also disturb conditions prevailing within a given soil type.

The work carried out in these experiments will be an endeavor to throw some light upon these aforementioned phenomena. No other work having come to hand pertaining to this phase of the soil biology problem it is hoped that the following experimentation will act as a stimulus for further constructive experimentation along this specific line.

Series I

The Associative Action of B. Subtilis and Zygorhynchus Vuilleminii with Moisture as the Limiting Factor.

The organisms chosen for experimentation were *Bacillus subtilis* and *Zygorhynchus Vuilleminii*. Both organisms are excellent representatives of the two groups of soil organisms. The soils used were the Penn clay loam and a second allotment of Norfolk sandy loam, the latter having a lime requirement of 2200 pounds CaO per acre. The soils were made up to an equal reaction basis by the use of $CaCO_3$. Dried blood and cottonseed meal respectively were used as the source of organic matter. The moisture boundaries are shown in the tables. The work was so outlined that the activities of the organisms in the presence of one source of organic matter and in two soil types are comparable. The incubation, inoculation, etc., were performed the same as in Part I.

Experiment XXXVIII. Table XXXVIII-A records the influence of varying amounts of moisture upon the activity of *B. subtilis*. Cottonseed meal was the ammonifiable material. The soil was the Penn clay loam. As will be seen by consulting the table very little activity was noted until 38 per cent of moisture was in the soil. From this point on however, increasing amounts of moisture result in a greater activity of the organism. The greater amount of moisture affording more free water for bacterial activity.

In Table XXXVIII-B one may again observe the activity of *Zygorhynchus Vuilleminii* under the above conditions of moisture. This organism, as previously noted is very active in a low moisture environment. The activity of this organism increases with the greater amounts of water until 43 per cent of moisture is at hand. Further increments had but slight effect either in depressing or accelerating the activity of this organism.

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When one observes the influence that moisture has upon the combined activities of these two organisms some very interesting data present themselves.

TABLE XXXVIII—A
THE AMMONIFYING EFFICIENCY OF B. SUBTILIS AS INFLUENCED BY MOISTURE

Lab. No.	% H_2O	Ammonia Accumulation			Increase over Check
		Mg. N.	Mg. N.	Av. Mg. N.	
1063-1064	18.70	7.98	7.00	7.49	4.87
1065-1066	23.70	8.36	6.65	7.50	4.42
1067-1068	28.50	7.56	7.70	7.63	4.51
1069-1070	33.20	7.26	8.40	7.83	4.71
1071-1072	38.30	10.36	10.50	10.43	7.31
1073-1074	43.10	14.97	.....	14.97	11.85
1075-1076	48.10	15.06	15.23	15.18	12.06
1077-1078	53.80	17.01	19.46	18.23	15.11
1079-1080	58.80	20.23	22.05	21.14	18.02
1081-1082	63.70	23.31	27.07	25.19	22.07
1082-1084	65.70	25.76	26.60	26.18	23.06

Table XXXVIII-C records the data and figure 5 shows graphically the associated and component activities of these organisms.

TABLE XXXVIII—B
THE AMMONIFYING EFFICIENCY OF ZYGORHYNCHUS VUILLEMINII AS INFLUENCED BY MOISTURE

Lab. No.	% H_2O	Ammonia Accumulation			Increase over Check
		Mg. N.	Mg. N.	Av. Mg. N.	
1085-1086	18.70	23.17	23.87	23.52	20.40
1087-1088	23.70	25.48	26.60	26.04	22.92
1089-1090	28.50	25.34	27.83	26.58	23.46
1091-1092	33.20	27.18	27.65	27.41	24.29
1093-1094	38.30	27.30	25.48	26.39	23.27
1095-1096	43.10	25.83	27.30	26.56	23.44
1097-1098	48.10	25.96	26.23	26.09	22.97
1099-1100	53.80	25.56	26.67	26.11	22.99
1101-1102	58.80	22.94	.....	22.94	19.82
1103-1104	63.70	25.60	24.85	25.23	22.11
1105-1106	65.70	26.74	26.74	26.74	23.62

A consultation of figure 5 shows very clearly that the curve resulting from the combined activities of these organisms is of a decided fungus nature and it seems safe to conclude that the fungus is the more dominating organism with the conditions at hand. It would also seem that the bacterium is more active at the lower moisture conditions when in combination with the fungus than at higher moisture conditions, as the sum of the individual activities corresponds more closely with the sum of the associated activities.

In soil having beyond 43 per cent of moisture, while both organisms are no doubt active, it seems indicative, judging from a consideration of the fungus nature of the curve representing the associated activities,

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that that organism was exerting a depressive action upon the activities of the bacterium.

TABLE XXXVIII—C
THE INFLUENCE OF MOISTURE UPON THE ASSOCIATED ACTIVITIES OF B. SUBTILIS AND ZYGORHYNCHUS VUILLEMINII

Lab. No.	% H_2O	Ammonia Accumulation			Inc. over Check	Theoretical Recovery	± Theoretical Recovery
		Mg. N.	Mg. N.	Av.Mg.N.			
1107-1108	18.70	24.57	25.97	25.27	22.15	25.27	3.12
1109-1110	23.70	29.75	26.50	28.12	25.00	27.34	2.34
1111-1112	28.50	27.30	.....	27.30	24.18	27.97	3.79
1113-1114	33.20	31.57	31.22	31.32	28.27	29.00	.73
1115-1116	38.30	32.27	32.27	32.27	29.15	30.58	1.43
1117-1118	43.10	33.11	32.20	32.65	29.53	35.29	5.76
1119-1120	48.10	29.47	32.76	31.11	27.99	35.03	7.04
1121-1122	53.80	31.27	31.36	31.31	28.19	38.01	9.82
1123-1124	58.80	32.13	.....	32.13	29.01	37.82	8.81
1125-1126	63.70	36.12	27.65	31.88	28.76	44.18	15.42
1127-1128	65.70	34.30	35.00	34.65	31.53	46.68	15.15

Experiment XXXIX. In this experiment the influence of moisture upon the activity of *B. subtilis* and *Zygorhynchus Vuilleminii* was de-

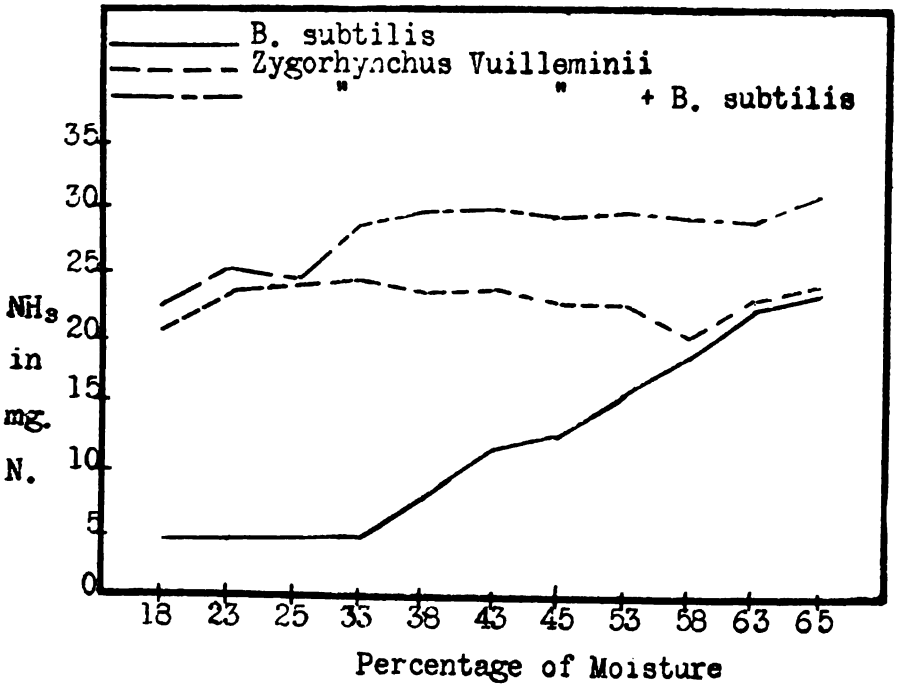

Fig. 5.—Diagram showing the associative action of *B. subtilis* and *Zygorhynchus Vuilleminii.*

termined as in the previous experiment using dried blood in place of cottonseed meal. The type of soil was the same. It is patent from a casual

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observation of Table XXXIX-A that the activity of *B. subtilis* in this type of soil was very feeble. This weak activity is not due to a poor inoculation or weak organism as can be seen by consulting Table XL-A to follow, both experiments receiving the same inoculation. Even with

TABLE XXXIX—A

THE INFLUENCE OF MOISTURE UPON THE AMMONIFYING EFFICIENCY OF B. SUBTILIS

Lab. No.	% H_2O	Ammonia Accumulation			Increase over Check
		Mg. N.	Mg. N.	Av. Mg. N.	
1129-1130	18.80	5.30	4.70	5.00	1.88
1131-1132	23.70	5.30	5.20	5.25	2.13
1133-1134	28.70	5.20	5.50	5.35	2.23
1135-1136	33.60	6.30	6.30	6.30	3.18
1137-1138	38.60	6.90	6.30	6.60	3.48
1139-1140	43.30	7.60	7.90	7.75	4.63
1141-1142	48.50	8.50	7.50	8.00	4.88
1143-1144	53.40	8.70	8.80	8.75	4.63
1145-1146	58.40	9.25	9.60	9.43	6.31
1147-1148	63.30	7.70	7.70	7.70	4.58

58 per cent of moisture in the system, affording an abundant supply of free water, only 6.31 mg. of nitrogen accumulated at the end of the incubation period.

TABLE XXXIX—B

THE INFLUENCE OF MOISTURE UPON THE AMMONIFYING EFFICIENCY OF ZYGORHYNCHUS VUILLEMINII

Lab. No.	% H_2O	Ammonia Accumulation			Increase over Check
		Mg. N.	Mg. N.	Av. Mg. N.	
1149-1150	18.80	5.40	6.10	5.75	2.63
1151-1152	23.70	11.80	10.60	10.20	7.08
1153-1154	28.70	13.00	....	13.00	9.88
1155-1156	33.60	11.40	14.70	13.05	10.93
1157-1158	38.60	15.30	14.20	14.75	11.68
1159-1160	43.30	16.50	17.30	16.90	13.78
1161-1162	48.50	14.80	16.10	15.45	12.33
1163-1164	53.40	14.90	14.50	14.70	11.58
1165-1166	58.40	10.92	11.63	11.27	8.15
1167-1168	63.30	8.20	7.20	7.70	4.58

The data recording the activity of *Zygorhynchus Vuilleminii* in the presence of dried blood under varying moisture conditions is tabulated in Table XXXIX-B. The maximum point of activity is reached with 43.30 per cent of moisture in the soil. Further increments had very little influence until 63.30 per cent of moisture was at hand.

This fact is in keeping with the previous experimentation. Also to be noted is the higher activity due to the higher acidity of the soil. In this case the acidity was equivalent to a lime requirement of 2200 pounds of CaO per acre.

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The data resulting from the combined activites of these organisms are shown in Table XXXIX-C. The data again suggest that in this soil type, with dried blood as the source of energy, that the fungus is again the dominant organism under nearly all the conditions of moisture.

At a moisture content of 18 per cent the sum of the combined activities is approximately equal to the component activities, indicating that at this figure both organisms are equally active.

TABLE XXXIX—C

THE INFLUENCE OF MOISTURE UPON THE ASSOCIATED ACTIVITIES OF B. SUBTILIS AND ZYGORHYNCHUS VUILLEMINII

Lab. No.	% H_2O	Ammonia Accumulation			Inc. over Check	Theoretical Recovery	± Theoretical Recovery
		Mg. N.	Mg. N.	Av.Mg.N.			
1169-1170	18.80	9.50	8.50	9.00	5.88	4.51	1.37
1171-1172	23.70	14.00	12.80	13.40	10.28	9.21	1.07
1173-1174	28.70	15.30	16.50	15.90	12.78	12.11	.67
1175-1176	33.60	18.40	18.50	18.45	15.33	14.01	1.32
1177-1178	38.60	16.00	25.10	20.55	17.43	15.16	2.27
1179-1180	43.30	17.00	17.30	17.15	14.03	18.41	4.38
1181-1182	48.50	16.50	.....	16.50	13.38	17.21	3.83
1183-1184	53.40	16.00	14.60	15.30	12.18	16.21	4.03
1185-1186	58.40	9.40	11.30	10.35	7.23	14.46	7.23
1187-1188	63.30	8.10	10.30	9.20	6.08	9.16	3.08

This phenomenon is also poignant until a point of moisture equal to 43 per cent is present. From here on, however, the activity of the fungus seems to be the predominating one as the curve (fig. 6) at these moisture contents is very "fungoid" in nature, falling off at 58 per cent and 63 per cent, respectively, as was characteristic of the fungus, whereas *B. subtilis* increased at the next to the highest moisture content and was depressed more gradually at 63 per cent.

A resumé of the data expressing the activities of these two organisms in the presence of dried blood and cottonseed meal suggests very strongly the possibility that variations in the moisture supply of the soil, or even, unequal distribution of the moisture already in the soil may influence to a considerable extent various groups of soil microörganisms. In both cases, e. g. in the presence of dried blood and cottonseed meal the activity of the fungus was accelerated by an increase in the moisture supply to a greater extent than was that of the bacterium. The activity of *B. subtilis* was greater in the presence of cottonseed meal than in the presence of dried blood. The same is true with *Zygorhynchus Vuilleminii*. The fungus, as well as the bacterium was more active at lower moisture contents in the presence of cottonseed meal than was the case where dried blood was used as the source of organic matter, which fact confirms the observations previously noted in Part III.

A consideration of the associated activities suggest an advantageous relationship existing in the presence of low amounts of moisture. Large

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quantities of water (40 to 50 per cent) seemed to favor the activity of the fungus, although it is very probable that both organisms are active. The trend of the plotted curves as compared with the component curves, indicates, in the main the above mentioned phenomena.

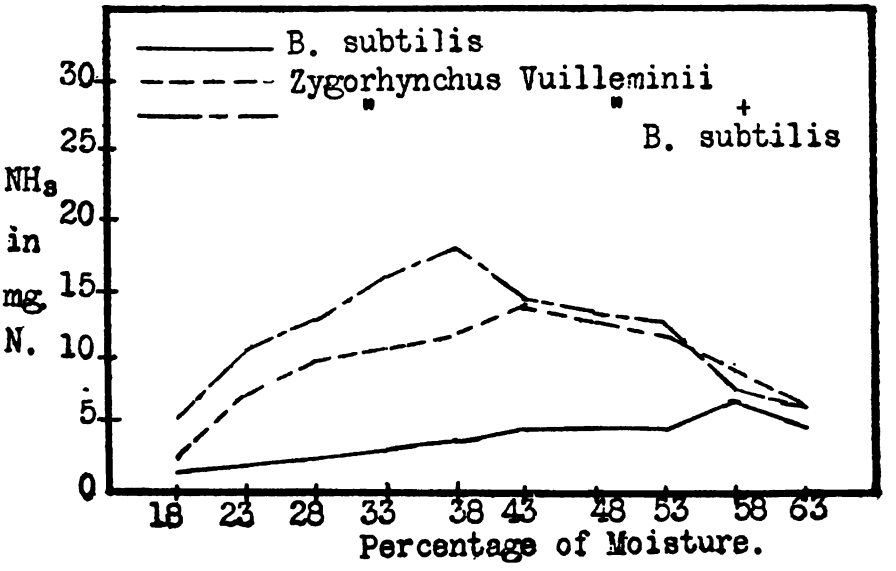

Fig. 6.—Diagram showing the influence of moisture on the associative activities of *B. subtilis* and *Zygorhynchus Vuilleminii.*

Experiment XL. A more open soil with its accompanying poorer chemical environment and increased oxygen supply might *a priori,* influence to a considerable degree, existing group relationships in a manner quite foreign to those occurring in a heavier soil. In order to throw some

TABLE XL—A

THE INFLUENCE OF MOISTURE UPON THE AMMONIFYING EFFICIENCY OF B. SUBTILIS

Lab. No.	% H_2O	Ammonia Accumulation			Increase over Check
		Mg. N.	Mg. N.	Av. Mg. N.	
1191-1192	11.80	8.10	8.90	8.50	6.00
1193-1194	15.80	13.20	13.20	13.20	10.70
1195-1196	19.80	15.50	22.60	19.05	16.55
1197-1198	24.20	22.40	20.10	21.25	18.65
1199-1200	29.70	28.70	29.80	29.25	26.75
1201-1202	34.10	10.50	8.80	9.68	7.15
1203-1204	38.60	8.20	8.40	8.30	5.80

light upon this phase of the problem the Norfolk sandy loam as previously described, was used, two sources of organic matter being employed.

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Table XL-A records the ammonifying efficiency of *B. subtilis* in this type of soil with dried blood as the source of organic matter. A glance at Table XL-A evidences the fact that both a change in the type of soil, as well as a greater supply of moisture, was very favorable to the ammonifying efficiency of this organism, an accelerated activity going hand in hand with increased amounts of moisture until a moisture content of 30 per cent or over double optimum conditions are at hand. A moisture content beyond this at once decimates the activity of this bacterium. It should be stated that the source of ammonfiable material was dried blood.

TABLE XL—B

THE INFLUENCE OF MOISTURE UPON THE AMMONIFYING EFFICIENCY OF ZYGORHYNCHUS VUILLEMINII

Lab. No.	% H_2O	Ammonia Accumulation			Increase over Check
		Mg. N.	Mg. N.	Av. Mg. N.	
1205-1206	11.80	7.30	7.80	7.55	5.05
1207-1208	15.80	8.40	7.50	7.95	5.45
1209-1210	19.80	9.60	15.00	12.30	9.80
1211-1212	24.20	11.00	12.70	11.60	9.10
1213-1214	29.70	10.40	10.90	10.65	8.15
1215-1216	34.10	4.40	5.20	4.80	2.30
1217-1218	38.60	4.50	4.50	4.50	2.00

Table XL-B tabulates the action of *Zygorhynchus Vuilleminii* with regard to a change of environment and the presence of varying moisture conditions. An observation of the table yields the information that there is a gradually accelerated activity of this fungus until double optimum conditions are reached. Beyond this point very little activity was noted.

TABLE XL—C

THE INFLUENCE OF MOISTURE UPON THE ASSOCIATED ACTIVITIES OF B. SUBTILIS AND ZYGORHYNCHUS VUILLEMINII

Lab. No.	% H_2O	Ammonia Accumulation			Inc. over Check	Theoretical Recovery	± Theoretical Recovery
		Mg. N.	Mg. N.	Av.Mg.N.			
1219-1220	11.80	13.10	13.20	13.15	10.65	11.05	.40
1221-1222	15.80	15.10	15.90	15.50	13.00	16.15	3.15
1223-1224	19.80	21.30	21.70	21.50	19.00	26.35	7.35
1225-1226	24.20	23.00	24.90	23.95	21.45	27.75	6.30
1227-1228	29.70	29.40	24.60	27.00	24.50	34.90	10.40
1229-1230	34.10	7.80	.....	7.80	5.30	9.45	4.15
1231-1232	38.60	7.00	7.80	7.40	4.90	7.80	2.80

The figures resulting from the combined activities of these organisms in this type of soil and this source of organic matter, dried blood, have been recorded in Table XL-C. As was the rule in the previous experiments curves for the activities of the organisms have been drawn and are presented in figure 7.

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The figures for the sum of the component and associated activities are approximately equal at the lowest moisture contents suggesting an advantageous relationship with very little if any antagonism. Conditions of 10 per cent and 15 per cent of moisture are conducive to an antagonistic action. The associated curve (fig. 7) is in the main bacterial in nature evidencing the point that the bacterium is the dominant power in all cases.

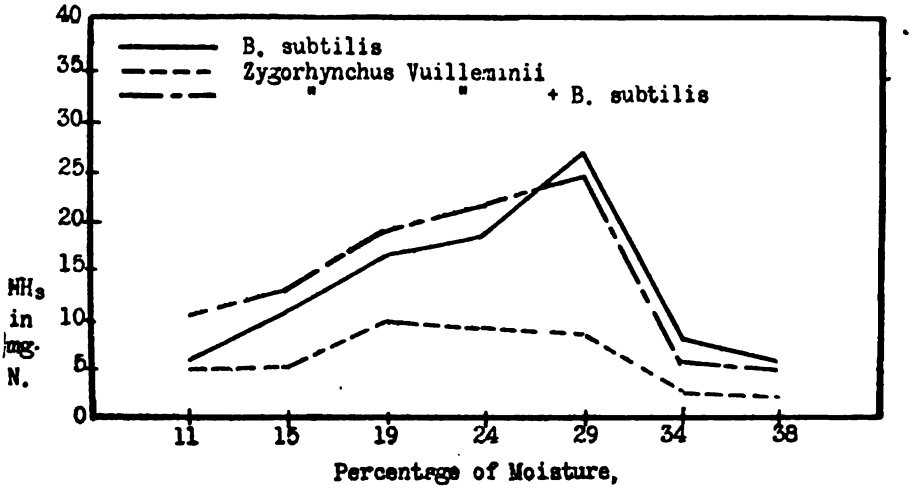

Fig. 7.—Diagram showing the influence of moisture on the associtive activities of *B. subtilis* and *Zygorhynchus Vuilleminii.*

However, as the sum of the associated activities, is generally speaking lower than that recorded by *B. subtilis* acting alone, one is forced to the conclusion that the fungus is depressing the activity of the bacterium. Just how great this depression is can not be accurately judged. A curve plotted to show the difference between the combined and theoretical recovery would also be bacterial in nature. It may be that the magnitude of depression is in this order. A bacterial count, with and without the presence of the fungus would probably clinch the magnitude of antagonism in a very decisive manner.

In order to bring out the influence of a change in organic matter upon the relationships of these two organisms as influenced by moisture conditions in this type of soil experiment XLI was made. Cottonseed meal was used in place of dried blood.

Experiment XLI. Table XLI-A is a record of both change in soil type and a variation of moisture conditions upon the ammonifying efficiency of *B. subtilis.* An observation of the data shows at once the beneficial influences due to a change of environment upon the activity of this fungus. A comparison with Experiment XXXVIII brings this out very

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forcefully. Whereas, in Experiment XXXVIII the maximum ammonification was approximately 10 mg. of nitrogen, the maximum in this experiment is nearer 50 mg. It is needless to say that both soils received the same inoculation. The response of the organism to additions of moisture is also more pronounced in this experiment.

TABLE XLI—A

THE INFLUENCE OF MOISTURE UPON THE AMMONIFYING EFFICIENCY OF B. SUBTILIS

Lab. No.	% H_2O	Ammonia Accumulation			Increase over Check
		Mg. N.	Mg. N.	Av. Mg. N.	
1233-1234	5.80	6.30	7.00	6.65	3.80
1235-1236	11.70	10.36	11.76	11.06	8.21
1237-1238	15.70	26.11	22.96	24.53	21.68
1239-1240	19.60	33.32	30.80	32.06	29.21
1241-1242	24.70	39.10	40.32	38.71	35.86
1243-1244	29.60	36.68	46.69	41.67	38.82
1245-1246	34.30	46.27	45.78	46.03	43.18
1247-1248	38.30	.....	46.34	46.34	43.49

In Table XLI-B the ammonifying efficiency of *Zygorhynchus Vuilleminii* under these new conditions has been recorded. In direct contradiction to all the previous experimentation, the activity of this organism persists with succeeding increments of water, the highest activity being noted with 40 per cent of moisture in the system. No explanation can be given for the phenomenon.

TABLE XLI—B

THE INFLUENCE OF MOISTURE UPON THE AMMONIFYING EFFICIENCY OF ZYGORHYNCHUS VUILLEMINII

Lab. No.	% H_2O	Ammonia Accumulation			Increase over Check
		Mg. N.	Mg. N.	Av. Mg. N.	
1249-1250	5.80	20.58	25.90	23.24	20.39
1251-1252	11.70	32.41	34.79	33.60	30.75
1253-1254	15.70	34.09	34.09	34.09	31.24
1255-1256	19.60	39.76	37.80	38.78	35.93
1257-1258	24.70	36.75	42.77	39.76	36.91
1259-1260	29.60	45.15	47.88	46.51	43.66
1261-1262	34.30	41.07	50.89	45.98	43.13
1263-1264	38.30	49.35	48.37	48.86	46.01

When the two organisms are combined and their activity measured by ammonia production we find antagonism operating even with 5 per cent of moisture in the soil. It is a clear case of the activity of the fungus being depressed by the bacterium. This phenomenon also holds true with the soil portions containing 10 per cent and 15 per cent of moisture (see Table XLI-C).

Further conclusions are not permissible as the figures for the individual activities are, in general, higher than the results obtained with the

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combined activities. It might be remarked, parenthetically, that the microscropical appearance of the fungus was very meager in the flasks where both organisms were acting in association.

TABLE XLI—C

THE INFLUENCE OF MOISTURE UPON THE ASSOCIATED ACTIVITIES OF B. SUBTILIS AND ZYGORHYNCHUS VUILLEMINII

Lab. No.	% H_2O	Ammonia Accumulation			Inc. over Check	Theoretical Recovery	± Theoretical Recovery
		Mg. N.	Mg. N.	Av.Mg.N.			
1265-1266	5.80	14.56	18.13	16.34	13.49	24.19	10.70
1267-1268	11.20	29.05	27.58	28.31	25.46	38.96	13.50
1269-1270	15.70	25.48	23.01	24.24	21.39	52.92	31.53
1271-1272	19.60	35.91	47.95	41.93	39.08	65.14	26.06
1273-1274	24.70	44.38	45.15	44.76	41.91	72.77	30.86
1275-1276	29.60	51.80	51.80	51.80	48.95	82.48	33.53
1277-1278	34.30	52.04	50.96	51.50	48.65	86.31	37.66
1279-1280	38.30	52.50	.....	52.50	49.65	89.50	39.85

As the fungus makes a very poor mycelial growth, however, this observation can not be taken as too prolix. The data from this experiment are shown graphically in figure 8.

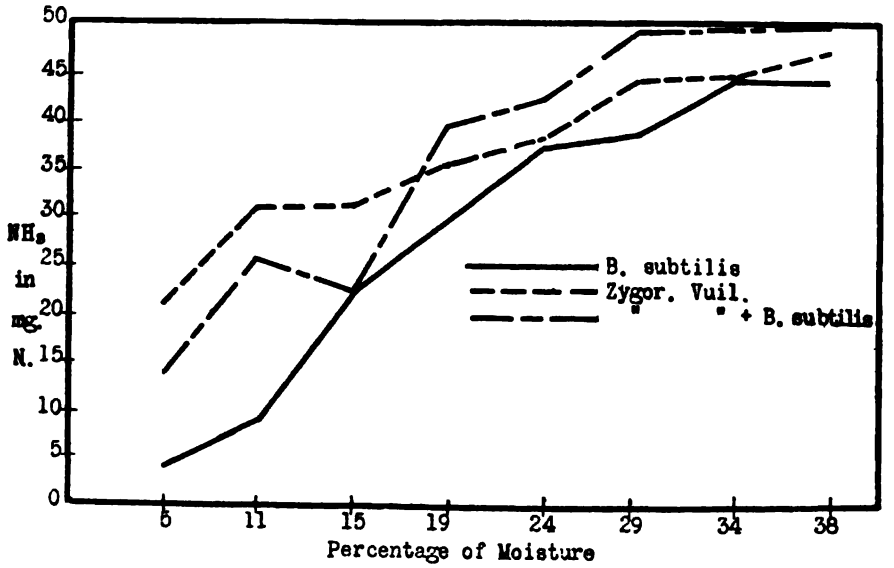

Fig. 8.—Diagram showing the influence of moisture on the associative activities of *B. subtilis* and *Zygorhynchus Vuilleminii*.

A recapitulation of the experimentation carried out in the sandy loam soil with both dried blood and cottonseed meal shows conditions prevailing that are just opposite from those recorded in the clay loam soil. In the experimentation carried out in the clay loam soil, it will be remembered that the fungus was the dominant organism always manifesting the

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greatest activity in the presence of both sources of organic matter. The bacterium was the depressing agent. In the experimentation carried out in the sandy loam soil the bacterium was the dominant organism under nearly all conditions having its activity depressed by the fungus.

A general summary of all the experimentation in this series suggests the facts that variations in the moisture contents of the soil may have considerable bearing in altering various biological relationships existing in them. At certain conditions of moisture more antagonistic action was exhibited than at others. In general the antagonistic action of the bacterium upon the fungus was more noticeable at lower concentration of moisture.

Standardizing the moisture factor and the source of organic matter, a change in the type of soil, was also potent in altering relationships between organisms.

The experimentation as carried out in this series indicates the following:

1. Variations in moisture may vary the group relations of soil microörganisms.
2. With the moisture and organic matter constant factors, a change in soil type may also change group relations.
3. The antagonistic action is shown more markedly at the lowest moisture contents.
4. *B. subtilis* and *Zygorhynchus Vuilleminii* both decompose cottonseed meal at lower moisture conditions than they do dried blood.
5. *Zygorhynchus Vuilleminii* was more active at lower percentages of moisture than was *B. subtilis*.
6. In the clay loam soil the fungus was the predominant organism, being depressed by the bacterium in its activities.
7. In the sandy loam soil the opposite proved true, i. e., the bacterium was the more active organism having its activity depressed by the fungus.

Series II

The Associative Action of B. Subtilis and Zygorhynchus Vuilleminii with Heat as the Limiting Factor

Heat as well as moisture is a factor that is of a variable nature. As the seasons come and go and even within the same seasons the changes in temperature of the atmosphere are often and varied. Necessarily these changes in the temperature of the atmosphere are followed by a change in the temperature of the soil.

These variations in the temperature of the soil are bound to exert some influence upon various groups of soil organism, some of which we know are favored by low temperatures, others by high and still other groups by intermediate temperature. The paucity of material bearing up-

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on this subject has stimulated the following investigation on this very interesting phase of the soil biology problem.

Experiment XLII. This experimentation was carried out with both types of soil. The acidity was adjusted as in Series I. Cottonseed meal was used as the source of organic matter; methods, etc., were the same as previously used. The temperatures employed were 6° to 8° C., 15° to 17° C., 22° to 25° C. and 30° C. Experiment XLII was carried out with the Penn clay loam. Table XLII records the single and combined activities of *B. subtilis* and *Zygorhynchus Vuilleminii* under various degrees of heat.

TABLE XLII

THE INFLUENCE OF TEMPERATURE UPON THE SINGLE AND ASSOCIATED ACTIVITIES OF B. SUBTILIS AND ZYGORHYNCHUS VUILLEMINII

Lab. No.	Temp. ° C.	Ammonia Accumulation			Increase over check	Biological Inoculation
		Mg. N.	Mg. N.	Av. Mg. N.		
1281-1282	6-8	4.27	4.13	4.20	—.20	Bacterium
1283-1284	15-17	8.12	8.33	8.22	3.72	"
1285-1286	22-25	9.10	10.08	9.59	5.04	"
1287-1288	30	18.06	17.15	17.60	13.10	"
1289-1290	6-8	3.92	4.20	4.06	.44	Fungus
1291-1292	15-17	18.34	18.83	18.59	14.09	"
1293-1294	22-25	29.54	27.86	28.70	24.70	"
1295-1296	30	.....	34.79	34.79	30.29	"
1297-1298	6-8	3.64	4.62	4.14	—.37	Bact. and Fungus
1299-1300	15-17	23.24	23.52	23.38	18.88	"
1301-1302	22-25	31.50	22.26	26.88	22.38	"
1303-1304	30	47.04	39.41	43.32	38.72	"

It will be noticed that at the lowest temperature employed no activity was recorded, either when the organisms were acting singly or in association. A rise in temperature to 15° C. results in activating both organisms. The fungus was the more extensively influenced at this temperature, its activity being 5 times as great as that recorded for the activity of the bacterium. A further increase in the temperature is again accompanied by an accelerated activity of both organisms. The fungus is again the most influenced by the rise in temperature. The same phenomenon also holds true at a temperature of 30° C.

Considering both organisms in association we find at 15° C. that 18.88 mg. of nitrogen accumulated. This is approximately the sum of the ammonia accumulation when these two organisms are working separately. It would seem that at this temperature both organisms are working independently of each other as no antagonistic action is noted.

On raising the temperature of 22° C. the sum of the ammonia accumulated from the component activities is 6.87 mg. less than the amount accumulated from the associated activities. Judging from the fact that the activity of the fungus while acting singly was greater than this, and further that the bacterium also was active it would seem that the

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bacterium was depressing the activity of the fungus at this temperature. This point is more favorably brought out if one consults figure 9, where the activity as measured by ammonia accumulation in milligrams of nitrogen has been plotted against the temperature.

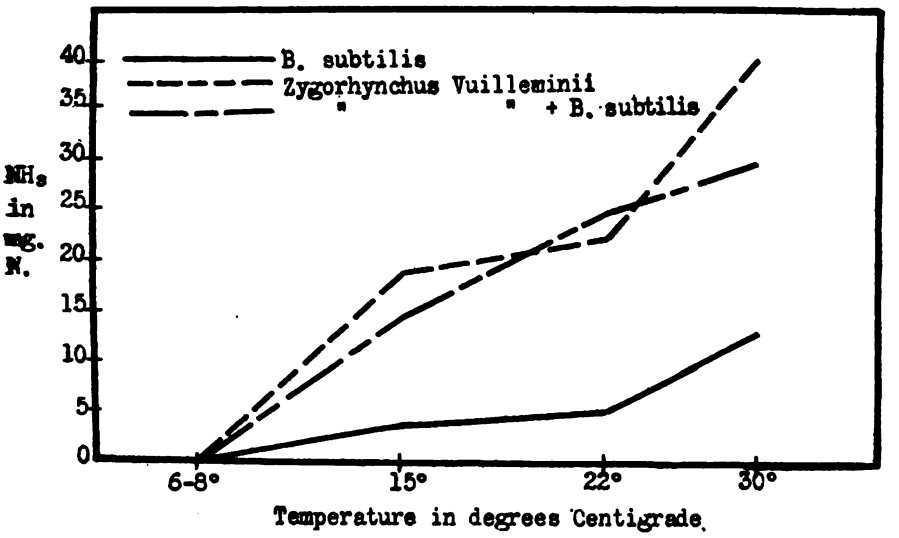

Fig. 9.—Diagram showing the influence of temperature on the associative activities of *B. subtilis* and *Zygorhynchus Vuilleminii*.

At 30° C. the phenomenon is different. The sum of the component activities would be theoretically, 43.39 mg. of nitrogen, whereas, the associative activities give us an accumulation of 38.72 mg. of nitrogen. At this temperature the activity of the fungus is equal to an accumulation of only 30 mg. of nitrogen and as the curve from 22° to 30° C. (fig. 9) tends to become highly bacterial in nature it is suggestive that the bacterium is again depressing the fungus in a larger measure than was exhibited at 22° C. The higher the temperature is raised the greater is the antagonistic action which seems to prevail.

Experiment XLIII is the same as the preceeding with a change in the type of soil. Both were inoculated at the same time so that all results are strictly comparable. As before, no activity is noted at 6° to 8° C.

In contradistinction to Experiment XLII, however, *B. subtilis* was more active in the lighter soil at 15° C. than was the fungus. This likewise holds true with regard to the respective activities in both soil types, with both sources of organic matter. The higher the temperature, the greater the activity, is the rule throughout this experiment.

When in associative action marked antagonism is to be seen. This phenomenon is present at 15° C. in this experiment, whereas at this temperature in the preceeding experiment no antagonism took place. Also,

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to be noted is the fact that antagonism becomes more marked as the temperature rises in this experiment.

TABLE XLIII

THE INFLUENCE OF TEMPERATURE UPON THE ASSOCIATIVE ACTIVITIES OF B. SUBTILIS AND ZYGORHYNCHUS VUILLEMINII

Lab. No.	Temp. ° C.	Ammonia Accumulation			Increase over check	Biological Inoculation
		Mg. N.	Mg. N.	Av. Mg. N.		
1305-1306	6-8	5.04	4.40	4.72	+.22	Bacterium
1307-1308	15-17	37.58	23.45	30.51	26.01	"
1309-1310	22-25	44.87	43.75	44.31	39.81	"
1311-1312	30	55.30	54.25	54.77	50.27	"
1313-1314	6-8	4.27	4.13	4.20	—.30	Fungus
1315-1316	15-17	22.61	23.84	23.23	18.73	"
1317-1318	22-25	33.32	34.09	33.70	29.20	"
1319-1320	30	56.84	57.04	56.94	52.44	"
1321-1322	6-8	4.06	4.41	4.23	—.27	Fungus and Bact.
1323-1324	15-17	32.27	34.02	33.14	28.64	"
1325-1326	22-25	50.67	45.64	48.15	43.65	"
1327-1328	30	57.75	57.67	57.71	53.21	"

At a temperature of 15° C. the sum of the component activities in milligrams of nitrogen would be 44.74, whereas the amount actually accumu-

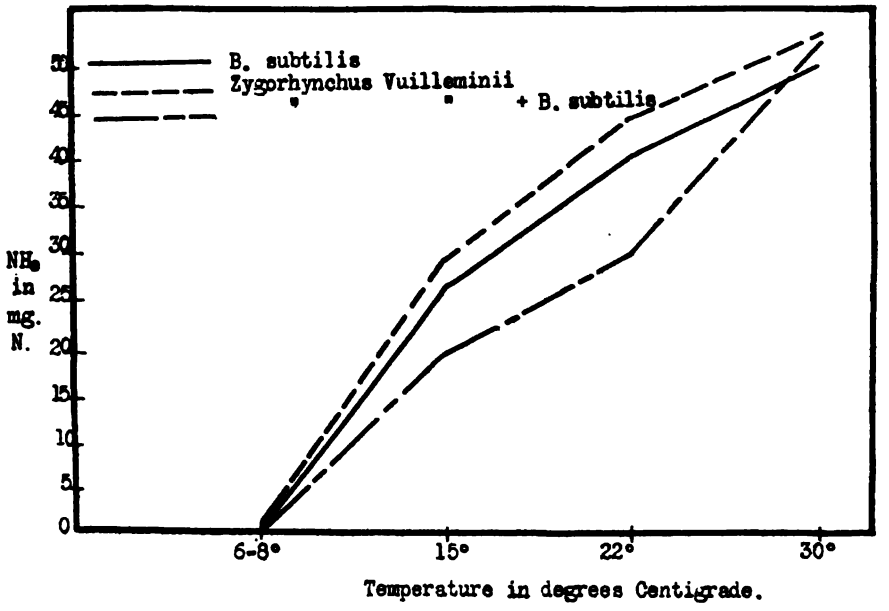

Fig. 10.—Diagram showing the influence of temperature on the associative activities of *B. subtilis* and *Zygorhynchus Vuilleminii*.

lating was only 28.64 mg., a difference of 15.10 mg. The difference at 22° C. is 25.63 mg. and at 30° C. it is 50.29 mg!

It is difficult to say whether the activity of the fungus in this type of soil has been entirely suspended by increased temperatures. It is sug-

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gested from a study of the data that the activity of the bacterium becomes antagonistic to the fungus at 15° C. with the fungus recovering its depressed state at 22° C., and becoming again depressed in its activity by the bacterium at 30° C.

The activity of these organism in this type of soil is graphically represented in figure 10.

In review, the work performed seems to indicate that temperature might easily alter group relations in the soil. This alteration seems to vary with the type of soil as the activity of *B. subtilis* at 15° C. as compared with *Zygorhynchus Vuilleminii* at this temperature, either alone or in associated action, differed widely in the two types of soil employed. This also applies to the experimentation carried out at 22° C. It is further opinioned that a change in organic matter might also cause changes entirely different from the above.

Series III

The Influence of Temperature upon the Ammonification of Dried Blood and Cottonseed Meal by the Flora of a Sandy Loam Soil

Experiment XLIV. Pertinent to the above data, are some figures accumulated from another line of investigation. In an attempt to determine the influence of nitrates upon the ammonifying power of a sandy loam soil the following experiments were made.

TABLE XLIV—A

THE AMMONIFICATION OF COTTONSEED MEAL AT 22° C.

Lab. No. A	Treatment	Ammonia Accumulation			±
		Mg. N.	Mg. N.	Av.Mg.N.	Increase
51-52	$NaNO_3$ = 100 lbs. per acre.....	42.50	39.80	41.15	.60
53-54	$NaNO_3$ = 300 lbs. per acre.....	43.00	48.60	45.80	4.05
55-56	$NaNO_3$ = 500 lbs. per acre.....	47.00	48.60	47.00	5.25
57-58	$NaNO_3$ = 700 lbs. per acre.....	58.80	.....	52.25	10.50
59-60	$NaNO_3$ = 1000 lbs. per acre.....	49.10	50.15	49.62	7.87
61-62	Nothing	41.40	42.10	41.75	....

Five-pound earthenware pots were filled with a weighed quantity of soil and treated with varying amounts of $NaNO_3$ as noted in the tables below. After having left the soil in contact with the chemical for two weeks under constant moisture conditions, portions of soil equal to 100 gm. of air-dry soil were drawn from the various pots and mixed with 3-gm. charges of dried blood and cottonseed meal. Moisture allowances were made for these materials. The portions were then inoculated in tumblers for 7 days at 22° C. and at the end of that time the ammonia was determined as in the previous work. Tables XLIV-A and XLIV-B record the data. An observation of these tables shows us that dried blood was ammonified to a smaller degree than cottonseed meal, the applications of nitrate, in general, causing a lessened accumulation of ammonia

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where dried blood was used when compared with the check treatment, and increasing the accumulation to a small degree where cottonseed meal was employed.

TABLE XLIV—B

THE AMMONIFICATION OF DRIED BLOOD AT 22° C.

Lab. No. A	Treatment	Ammonia Accumulation			
		Mg. N.	Mg. N.	Av.Mg.N.	Increase
63-64	$NaNO_3$ ⇔ 100 lbs. per acre.....	41.50	41.30	41.40	— 5.25
65-66	$NaNO_3$ ⇔ 300 lbs. per acre.....	36.70	36.50	36.60	—10.05
67-68	$NaNO_3$ ⇔ 500 lbs. per acre.....	44.10	44.10	44.10	— 2.55
69-70	$NaNO_3$ ⇔ 700 lbs. per acre.....	35.00	36.70	35.85	—10.80
71-72	$NaNO_3$ ⇔ 1000 lbs. per acre.....	33.40	36.10	34.75	—11.90
73-74	Nothing	47.00	46.30	46.65	.....

Experiment XLV. Upon repeating the above experiment as confirmatory work the thermostat of the incubating chamber became disarranged and induced a temperature of 15° to 17° C. for the major portion of the experiment, with the striking results shown in Table XLV-A.

Tables XLV-A and XLV-B record the experimentation carried out at 15° to 17° C.

TABLE XLV—A

THE AMMONIFICATION OF COTTONSEED MEAL AT 15° TO 17° C.

Lab. No. A	Treatment	Ammonia Accumulation			
		Mg. N.	Mg. N.	Av.Mg.N.	Increase
87-88	$NaNO_3$ ⇔ 100 lbs. per acre.....	43.90	37.00	40.45	— 8.30
89-90	$NaNO_3$ ⇔ 300 lbs. per acre.....	37.60	41.70	39.60	— 9.15
91-92	$NaNO_3$ ⇔ 500 lbs. per acre.....	33.57	28.10	30.83	—17.92
93-94	$NaNO_3$ ⇔ 700 lbs. per acre.....	33.00	32.50	32.75	—16.00
95-96	$NaNO_3$ ⇔ 1000 lbs. per acre.....	30.90	.....	30.90	—17.85
97-98	Nothing	47.70	50.30	48.75	.....

Dried blood was ammonified nearly to twice the extent as was the case in the first experiment, nitrate again causing a lessened accumulation of ammonia. Cottonseed meal on the other hand was ammonified to a smaller extent than in the previous experiment.

Also to be noted is the fact that the ammonification of cottonseed meal was not enhanced as in the previous experiment by the presence of nitrate of soda, but that the accumulation was lessened in a manner similar to the results obtained where dried blood was employed.

It has been pointed out in the preceeding pages that soil fungi seem to be relatively poor ammonifiers at low temperatures, whereas the temperature ranges for the activity of bacteria are quite wide, being somewhat lower than that exhibited by the fungi at hand. It has also been shown that fungi prefer materials of a carbohydrate nature for their activity. The following explantion of the above phenomenon suggests itself.

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At 22° C. the fungi and bacteria were acting together in the degradation of the organic matter, with an antagonistic action taking place between these two groups. It is also suggested that at 22° C., in the presence of dried blood, that the bacteria were the predominant flora, whereas, in the presence of cottonseed meal the fungi could be the dominant factor. When the nitrate of soda was applied to a soil having, *a priori*, a bacterial flora, the resulting effect was to cause a decrease in the ammonia accumulation, whereas, when this chemical was applied to a flora, *a priori*, namely of a fungus nature these organisms were the most influenced.

TABLE XLV—B

THE AMMONIFICATION OF DRIED BLOOD AT 15° TO 17° C.

Lab. No. A	Treatment	Ammonia Accumulation			
		Mg. N.	Mg. N.	Av.Mg.N.	Increase
75-76	$NaNO_3$ = 100 lbs. per acre.....	77.40	.....	77.40	— 4.50
77-78	$NaNO_3$ = 300 lbs. per acre.....	76.10	77.30	76.70	— 5.20
79-80	$NaNO_3$ = 500 lbs. per acre.....	77.10	71.10	74.10	— 7.80
81-82	$NaNO_3$ = 700 lbs. per acre.....	55.80	64.70	60.25	—21.65
83-84	$NaNO_3$ = 1000 lbs. per acre.....	59.30	63.60	61.45	—20.45
85-86	Nothing	81.40	82.40	81.90	.....

At 15° to 17° C., a temperature unfavorable to the activity of soil fungi was at hand. It can be conceived, then, that the bacteria, released to a large extent from their competition with the fungi would exhibit an enhanced effect, as was manifested in Table XLV-B. The fungi being depressed by the lower temperature could not give us our maximum ammonification of cottonseed meal. The flora would then probably become bacterial in nature. Evidence of this is presented when one regards the depressing influence of the nitrate of soda, this phenomenon being in correlation with the influence of nitrate upon the ammonification of dried blood.

This phenomenon may also have a bearing upon the increased numbers of bacteria in frozen soil. Would not the bacteria be able to multiply faster when released from any serious competition, as for example the soil fungi, assuming that the latter became relatively inactive at comparatively low temperatures as the work in Part IV indicates? If this should be the case the increased multiplication at medium low temperatures would account in a large measure for the higher bacterial numbers reported in frozen soils, as the increased numbers found at medium temperatures would be present, either in an active or spore state at the very low temperatures.

General Summary

The experimental data above submitted constitute a series of systematic researches dealing with the factors that may influence the activity of soil fungi. Several of the salient facts which have come to light during the course of the above investigations are highly suggestive and demand a word of comment with respect to their general significance.

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First, with regard to the activities of the different organisms tested, it was found that the type of soil, as well as the quality of the organic matter regulated the activities of these organisms. It appears, judging from the standpoint of pure cultures that every organism will do best with a definite combination of soil and organic matter. As a general rule, vegetable matter of high quality was conducive to the greatest activity of these organisms. Dried blood was conducive to an accelerated action of two organisms.

In the second place, the experimentation concerning the activity of soil fungi as influenced by the mechanical composition of the soil indicates a wide divergency of response to increased oxygen supply. The data intimate that some fungi would be greatly benefited by an increased oxygen pressure, others but very little and still others express relation gradient between high and low oxygen pressures.

Of possible importance was the phenomenon expressed by the fungi with regard to a bettered chemical environment. The materials beneficial to one group or species may be detrimental to the other groups of organisms, suggesting a possible alteration of group relations among the microbes in the soil.

Concerning the influence of moisture upon the activity of soil fungi the results indicate that some fungi require moisture conditions that are at a variance to the requirements of other fungi. For instance, two of the forms studied demanded a dry medium for their activity, two were the most active under optimum soil moisture conditions and one required a high moisture environment for its best activity.

In the studies carried out with the influence of temperature upon the activities of these organisms a very narrow temperature range was observed, with an optimum temperature of about 30° C. for all the species studied.

In the studies carried out with the associated activities of bacteria and fungi, one finds some very interesting data, pointing to the possible changes among group relations of soil microörganisms due to food, moisture, temperature and soil conditions.

However, the data recorded must not be taken too prolixly. The limitations of the methods employed must be fully realized. Sterilized soil is somewhat different from fresh field soil. By sterilizing the soil plant-food is made available as shown by us (3) in another paper. Toxic bodies are also created as shown by Pickering (31, 32) and others (37). It is thought that the excess food offered in the form of organic matter for the ammonification process entirely alleviates any possible beneficial influences that sterilization might give to the soil by releasing plant-food.

Any toxic bodies which may have been created due to sterilization are in all probability a factor to be reckoned with. The soils used are only two of the many existing types and the fungi and bacterium tested typify

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only potent leaders of two important groups of soil microörganisms. All these facts must be taken into consideration in drawing final conclusions from this work.

The results are very suggestive, however, and it is hoped that the material herein submitted will act as a stimulus for further constructive experimentation in this new branch of soil science.

In conclusion, the author's gratitude is due Dr. M. T. Cook, Prof. J. P. Helyar, Prof. A. W. Blair, and Mr. C. R. Fellers for suggestions and aid in carrying out this work, and especially to Dr. J. G. Lipman under whom the work of this thesis has been concluded.

Literature Cited

(1) Ames, Adeline
1915. The temperature relations of some fungi causing storage rots. *In* Phytopathology, v. 5, no. 1, p. 11-19.

(2) Butkewitsch, W.
1903. Umwandlung der Eiweissstoffe durch die niederen Pilze im Zusammenhange mit einigen Bedingungen ihre Entwickelung. *In* Jahrb. Wiss. Bot. [Pringsheim], Bd. 38, p. 147.

(3) Coleman, D. A., Lint, H. C., and Kopeloff, N.
1916. Can soil be sterilized without radical alteration? *In* Soil Sci. v. 1, no. 3, p. 259-271.

(4) Coleman, L. C.
1908. Untersuchungen über nitrification. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 20, p. 401, 484.

(5) Conn, H. J.
1910. Bacteria of Frozen Soil I. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 28, p. 422-428.
1911. Bacteria of Frozen Soil II. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 32, p. 70-79.
1914. Bacteria of Frozen Soil III. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 42, p. 510-519.

(6) Cook, M. T., and Taubenhaus, J. J.
1911. The relation of parasitic fungi to the content of the cells of the host plant. *In* Del. Agr. Exp. Sta. Bul. 91, p. 11.

(7) Engberding, D.
1909. Vergleichende Untersuchungen über die Bakterienzahl in Ackerboden in ihrer Abhangigkeit von aüssern Einflussen. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 23, p. 569-642.

(8) Fischer, H.
1909. Bakteriologische-chemishe Untersuchungen. *In* Landw. Jahrb., Bd. 38, p. 358.

(9) Faelli, G.
1904. Keimgehabt des Humusbodens. Ref. *in* Centbl. Bakt. [etc.], Abt. 2, Bd. 14, p. 423.

(10) Goddard, H. N.
1913. Can fungi in agricultural soil assimilate free nitrogen? *In* Bot. Gaz., v. 56, no. 4, p. 249-304.

Digitized by Google

(11) GERLACH, M., and VOGEL, J. H.
1901. Uber eiweissbildende Bakterien. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 7, p. 614.
(12) HALL, A. D., MILLER, N. H. J., and GININGHAM, C. T.
1908. Nitrification in acid soils. *In* Proc. Roy. Soc. [London], S. B 80, no. B 539, p. 196-212.
(13) JENSEN, H.
1898. Bieträge zur Morphologie und Biologie der Denitrifikateonsbakterien. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 4, p. 402.
(14) KAPPEN, H.
1910. Uber die Zersetzung das Cyanamides durch Pilze. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 26, p. 633.
(15) KOPELOFF, NICHOLAS
1916. The inoculation and incubation of soil fungi. *In* Soil Sci., v. 1, no. 4, p. 381-403.
(16) KOPELOFF, NICHOLAS.
1916. The effect of soil reaction on ammonification by certain soil fungi. *In* Soil Sci., v. 1, no. 6, p. 541-574.
(17) KOPELOFF, N., LINT, H. C., and COLEMAN, D. A.
1915. New methods in soil protozology. *In* Science, n. s., v. 57, no. 1078, p. 284-286.
(18) LINT, H. C., and COLEMAN, D. A.
1916. Sources of error in soil biological analyses. *In* Soil Sci. (soon to appear).
(19) LIPMAN, C. B., and BURGESS, P. S.
1914. Studies on ammonification in soils by pure cultures. *In* Univ. Cal. Pub. Agr. Sci., v. 1, no. 7, p. 141-172.
(20) LIPMAN, C. B., and SHARP, L. T.
1915. Effect of moisture content of a sandy soil on its nitrogen-fixing power. *In* Bot. Gaz., v. 59, p. 402-406.
(21) LIPMAN, J. G.
1911. Suggestions concerning the terminology of soil bacteria. *In* Bot. Gaz., v. 51, p. 454.
(22) LIPMAN, J. G., and BROWN, P. E.
1908. Notes on methods and culture media. *In* N. J. Agr. Exp. Sta. 29th Ann. Rpt., p. 129.
(23) LIPMAN, J. G., and BROWN, P. E.
1908. Media for the quantitative estimation of soil bacteria. *In* N. J. Agr. Exp. Sta. 29th Ann. Rpt., p. 132.
(24) LIPMAN, J. G., and BROWN, P. E.
1908. Moisture conditions affecting the formation of ammonia, nitrites and nitrates. *In* N. J. Agr. Exp. Sta. 29th Ann. Rpt., p. 105.
(25) LÖHNIS, FELIX.
1914. Ammonification of cyanamid. *In* Ztschr. Garung's Physiol., Bd. 5, No. 1, p. 16-25.
(26) MCLEAN, H. C., and WILSON, G. W.
1914. Ammonification studies with soil fungi. N. J. Agr. Exp. Sta. Bul. 270, 39 p., 1 pl.
(27) MARCHAL, E.
1893. La production l'ammonique dans la terre par les microbes. *In* Bul. Acad. Roy. Belgique, s. 3, v. 25, p. 727-771.
(28) MARSHALL, C. E.
1912. Microbiology. P. Blakiston's Son & Co., Philadelphia, p. 154.

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(29) MÜLLER, M.
1903. Uber das Wachstum und die Lebenstätigkeit von Bakterien sowie den Ablauf fermentation Prozesse bei Niederer Temperatur unter spezieller Berücksichtigung des Fleisches als Nahrungmittel. *In* Arch. Hyg., Bd. 47, p. 126-193.

(30) MUNTZ, A., and COUDON, H.
1893. La fermentation ammonicale de la terre. *In* Ann. Agron., t. 19, no. 5, p. 209-216.

(31) OUDEMANS, A. C., and KONING, C. J.
1902. Prodrome d'une flore Mycologique obtenue par la culture sur gelatine prepare de la pres de Bussum. *In* Arch. Néerland Sci. Exact. et Nat., v. 2, p. 266.

(32) PICKERING, S. U.
1908. Studies on germination and plant growth. *In* Jour. Agr. Sci., v. 2, p. 411-434.

(33) PICKERING, S. U.
1908. The action of heat and antiseptics. *In* Jour. Agr. Sci., v. 3, p. 32-54.

(34) RAMANN, E.
1905. Bodenkunde, 2 Auff. Berlin. J. Springer, p. 119-125.

(35) RUSSELL, E. J., and HUTCHINSON, H. B.
1909. The effect of partial sterilization upon the production of plant-food, I. *In* Jour. Agr. Sci., v. 3, p. 111-144.

(36) RUSSELL, E. J., and HUTCHINSON, H. B.
1913. The effect of partial sterilization of soil on the production of plant-food, II. The limitation of bacterial numbers in normal soils and its consequences. *In* Jour. Agr. Sci., v. 5, pt. 2, p. 152-221.

(37) SCHREINER, O., and LATHROP, E. C.
1912. The chemistry of steam-heated soils. *In* U. S. Dept. Agr. Bur. Soils, Bul. 89.

(38) TRAAEN, A. E.
1914. A study of soil fungi from Norway. *In* Nyt. Mag. Naturvidensk, v. 52, no. 1-2, p. 19-121.

(39) WAKSMAN, S. A., and COOK, R. C.
1916. Incubation studies with soil fungi. *In* Soil Sci., v. 1, no. 3, p. 275-284.

(40) WAKSMAN, S. A.
1916. Soil fungi and their activities. *In* Soil Sci. (soon to appear).

(41) WOLLNY, E.
1897. Die Zersetzung der organischen Stoffe, Heidelburg. C. Winter, 1897.

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THE EFFECT OF SOME MANGANESE SALTS ON AMMONIFICATION AND NITRIFICATION[1]

By

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The relation of manganese to plant growth has attracted some attention in times past among plant physiologists, but it has been generally conceded that although it occurs in all plants, it does not serve directly as a nutritive element. Under artificial conditions, it has been possible to grow perfect plants in the entire absence of manganese, but applications of manganese salts have frequently been found to bring about increases in crop yields. The reasons for such beneficial effects have not been ascertained, but it has been assumed that the action was a stimulative one.

Some recent experiments by Skinner and Sullivan (6) demonstrated the fact that manganese acts in various ways as a fertilizer. It is often without influence, occasionally injurious, but usually beneficial, its effect depending apparently upon the composition and character of the soil. The oxidation in soils under treatment with manganese salts was also studied and it was found that an increase in oxidation and growth frequently occurred in aqueous extracts of poor, unproductive soils, but while oxidation was increased in fertile soils, growth was decreased, the plants showing indications of excessive oxidation. Field experiments showed practically no effect from the manganese salts, but the soil was acid, a condition which may have accounted to a considerable degree for the nature of the results.

It is suggested that when the action of manganese is beneficial, "it is probably due (1) to the increased oxidation produced in the plant roots whereby the plant is stimulated to greater activity and to increased absorption of the material useful for its growth and general metabolism; (2) *to the stimulation of the activity of microörganisms in the soil;* (3) to an increased oxidation within the soil." (6, p. 28).

The same authors also suggest that when large applications of manganese have been found to be injurious, the injury is undoubtedly due to the "excessive stimulation and excessive oxidation in microörganisms and in the plant, with a resulting change in the biochemical activities of plant and microörganisms and in the conditions of inorganic and organic soil constituents, the ultimate result of which change is injurious to the growing crop." (6 p. 29).

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It is apparent, therefore, that the effect of certain manganese salts on soil bacteria should be determined, as it may be that the effect of the compounds on the crop grown is due to the influence on soil bacteria.

Are necessary bacteriological changes encouraged or restricted by manganese salts in small amounts? What bacteriological processes are affected and how are they influenced? What relation to the effects on crop yields are the effects on certain bacteria? These are a few of the questions which arise in the minds of those who consider the bacterial side of the problem of manganese fertilization.

Kelley (2) tested ammonification and nitrification in Hawaiian soils high and low in maganese and found that nitrification took place more rapidly in the manganese soils, while ammonification was about the same in the manganese soils as in the normal soils. He attributed the greater nitrification in the manganese soils to their better physical condition and hence better and greater oxidation. The conclusion reached was that manganese does not interfere with the growth of the nitrifying or ammonifying bacteria. Later experiments (3) by the same author confirmed these results.

Montanari (5) determined the effect of some manganese salts on nitrifying bacteria and he found that 0.5 gm. of MnO_2, 0.1 gm. of $MnSO_4$ and 0.5 gm. of $MnCO_3$ in 50 gm. of soil each increased the nitrification of ammonium sulfate.

Leoncini (4) recently studied the effect of manganese oxide on nitrification and found that applications at the rate of 0.035-2.2 per cent favored nitrification, but larger amounts had no influence.

No further experiments from the bacteriological standpoint have been carried out as far as the authors are aware.

It was the purpose of the following experiments to throw some light on the problem from the bacteriological standpoint. It was planned to study the effect of certain manganese salts on ammonification and nitrification, using a normal field soil as the basis of the test.

The results obtained in the work are included in the following pages. They are necessarily somewhat preliminary in nature, dealing as they do with merely one type of soil, but the conclusions reached may be of more general application than is anticipated, and at any rate they will apply to soils of similar chemical and mechanical composition.

The Plan of the Experiment

The soil used in the tests was a Carrington clay loam, containing 0.1732 per cent of manganese as determined by the colorimetric method described by Hillebrand (1). A large sample of this soil was secured, air-dried, sieved thoroughly, mixed and stored for use.

Various series of tests were carried out using manganese sulfate, manganese chloride, manganese nitrate and manganous oxide in varying

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amounts to ascertain their effect on the ammonification of dried blood and on the nitrification of ammonium sulfate.

In the ammonification tests 5 gm. of dried blood were added to 100 gm. of the air-dried soil in tumblers and thoroughly stirred in, the additions of manganese salts made as called for, the moisture content adjusted to the optimum for the soil, 12 c.c. of additional water being supplied on account of the organic matter used, and the tests incubated for 6 days. The ammonia was distilled off by the magnesium oxide method.

In the nitrification experiments 100 mg. of ammonium sulfate was added to the air-dry soil in tumblers, the manganese salts supplied, 10 c.c. of an infusion of a fresh soil added, the moisture content adjusted to the optimum, and the tests were incubated for four weeks. The moisture content was kept up during the incubation period by adding water to weight every 10 days.

All of the tests were incubated at room temperature.

Series I

The Effect of $MnCl_2$ on Ammonification

The effect of manganese chloride on the ammonification of dried blood was tested in this series, applications of 0.1 gm., 0.5 gm., 1.0 gm., 3.0 gm., and 5.0 gm. of the salt being made to duplicate portions of 100 gm. of soil. The results appear in Table I.

TABLE I

THE EFFECT OF $MnCl_2$ ON AMMONIFICATION

No.	Treatment	Ammonia Mg. N.	Average Mg. N.
1	Check	170.29	
2	Check	174.01	172.15
3	0.1 gm. $MnCl_2$	159.60	
4	0.1 gm. $MnCl_2$	156.74	158.17
5	0.5 gm. $MnCl_2$	102.17	
6	0.5 gm. $MnCl_2$	110.66	106.42
7	1.0 gm. $MnCl_2$	73.80	
8	1.0 gm. $MnCl_2$	70.12	71.96
9	3.0 gm. $MnCl_2$	25.09	
10	3.0 gm. $MnCl_2$	22.42	23.76
11	5.0 gm. $MnCl_2$	0	
12	5.0 gm. $MnCl_2$	0	0

It is apparent from an examination of this table that the chloride of manganese exerted a pronounced depressing action on the ammonification of dried blood. The depression was considerable when 0.1 gm. was used and increased rapidly with the increasing additions until with the 5.0-gm. quantity no ammonification whatever occurred.

If manganese chloride is able to bring about any increases in ammonification, it is evident that smaller amounts than those used here must be employed, at least on this soil. This result is not surprising as the smallest amount applied in this series would represent the use of 2000 pounds

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per acre, which would be a very large addition. It was deemed advisable, however, to ascertain the upper limit of safety in the use of the salt from the standpoint of its effect on ammonification which represents, of course, the decomposition of organic matter. The largest amount of manganese chloride which may be applied to the soil without depressing the production of available plant-food is evidently less than 2000 pounds per acre.

Series II

The Effect of $MnCl_2$ on Ammonification (Continued)

This series was planned to supplement the preceeding in the effort to determine the effect of manganese chloride on ammonification. The amounts of the salt added were very much smaller than those used in the first test, being gradually increased up to the smallest amount used in that case.

The applications used and the results secured are given in Table II.

TABLE II

THE EFFECT OF $MnCl_2$ ON AMMONIFICATION—(Continued)

No.	Treatment	Ammonia Mg. N.	Average Mg. N.
1	Check	167.19	
2	Check	174.45	170.82
3	0.005 gm. $MnCl_2$	176.77	
4	0.005 gm. $MnCl_2$	170.10	173.44
5	0.01 gm. $MnCl_2$	175.51	
6	0.01 gm. $MnCl_2$	lost	175.51
7	0.03 gm. $MnCl_2$	164.68	
8	0.03 gm. $MnCl_2$	165.20	164.94
9	0.05 gm. $MnCl_2$	148.14	
10	0.05 gm. $MnCl_2$	157.62	152.88
11	0.07 gm. $MnCl_2$	151.53	
12	0.07 gm. $MnCl_2$	156.17	153.85
13	0.09 gm. $MnCl_2$	144.96	
14	0.09 gm. $MnCl_2$	151.97	148.47

On examining this table, it appears that 0.005 gm. and 0.01 gm. of $MnCl_2$ gave slight increases in ammonification, but the larger additions all depressed the amount of ammonia produced, the depression increasing with increasing additions.

There was a very slight difference in the ammonia production where the 0.05 gm. and the 0.07 gm. of the chloride were added, but the variations in the duplicate determinations would account for the results secured. It is probable that the higher result in the case of the 0.05-gm. treated soil should be considered, in which case the continuous depression in ammonification with increasing amounts of the chloride would be definitely shown. It is apparent, however, that the differences were slight and the conclusion seems warranted that increasing the amounts of the chloride increases the depression in ammonification.

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The small increases produced by the two smallest applications of the chloride can hardly be considered conclusive. The differences were too small, and while the results indicate a slight beneficial effect from these amounts of manganese chloride on ammonification, it might be that the results should be interpreted to mean the absence of any effect. The nitrification results may indicate how these results should be interpreted, that is, whether the increases noted should be considered definite or not.

Applications of manganese chloride to the soil, therefore, in amounts not exceeding 200 pounds per acre of two million pounds of surface soil had no depressing action on ammonification and gave indication of a slight beneficial effect. Larger amounts of the salt, however, brought about depressions in ammonia production and these depressions gradually increased as the applications increased.

Series III

The Effect of $MnCl_2$ on Nitrification

This series was arranged to test the effect of manganese chloride on nitrification. The amounts of the manganese salt applied to the soil were the same as those used in Series I.

The treatments and results are given in Table III.

TABLE III

THE EFFECT OF $MnCl_2$ ON NITRIFICATION

No.	Treatment	Nitrate Mg. N.	Average Mg. N.
1	Check	6.250	
2	Check	4.166	5.208
3	0.1 gm. $MnCl_2$	6.250	
4	0.1 gm. $MnCl_2$	0.586	3.418
5	0.5 gm. $MnCl_2$	2.344	
6	0.5 gm. $MnCl_2$	3.125	2.735
7	1.0 gm. $MnCl_2$	0.879	
8	1.0 gm. $MnCl_2$	0.586	0.733
9	3.0 gm. $MnCl_2$	0.220	
10	3.0 gm. $MnCl_2$	0.220	0.220
11	5.0 gm. $MnCl_2$	trace	
12	5.0 gm. $MnCl_2$	trace	trace

On examining the results in the table, it becomes evident that the chloride of maganese depressed nitrification to a pronounced extent. The depression was apparent with the smallest amount, 0.1 gm., although the duplicates in this case were not entirely satisfactory, and became gradually greater until, with the largest addition of the chloride, no nitrification whatever occurred in the soil.

These results checked exactly those secured in Series I for ammonification. The depression with the smallest amount was found in both cases and with the largest addition neither ammonification nor nitrification occurred in the soil. From the results thus far it would seem that the two processes were similarly influenced by the use of maganese chloride.

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It remains for the following series to determine whether the effects of the smaller applications on the two processes were the same.

Series IV

The Effect of $MnCl_2$ on Nitrification (Continued)

This series was a continuation of the preceeding, but smaller additions of the manganese chloride were used. The amounts applied were the same as those used in Series II.

On turning to Table IV which gives the applications and the results secured, it is found that conclusions are difficult to reach. The variations among the average results were less than the differences among the duplicates in many cases and definite conclusions should not be drawn. It seems that all the amounts of the salt used here increased slightly the nitrifying power of the soil. If it is deemed, therefore, that the differences were large enough to permit of such a conclusion, it would be evident that the use of 0.09 gm. of manganese chloride would not only be safe, but would actually increase slightly the nitrifying power of the soil. On the other hand, if the results are not considered as showing any increases, at least they show no depressions and it would seem that any of the amounts used would not be injurious to the nitrifying organisms.

TABLE IV

THE EFFECT OF $MnCl_2$ ON NITRIFICATION—(Continued)

No.	Treatment	Nitrate Mg. N.	Average Mg. N.
1	Check	13.16	
2	Check	12.50	12.83
3	0.005 gm. $MnCl_2$	13.88	
4	0.005 gm. $MnCl_2$	15.44	14.66
5	0.01 gm. $MnCl_2$	12.50	
6	0.01 gm. $MnCl_2$	15.24	13.87
7	0.03 gm. $MnCl_2$	13.16	
8	0.03 gm. $MnCl_2$	13.84	13.50
9	0.05 gm. $MnCl_2$	13.84	
10	0.05 gm. $MnCl_2$	13.16	13.50
11	0.07 gm. $MnCl_2$	15.44	
12	0.07 gm. $MnCl_2$	13.84	14.64
13	0.09 gm. $MnCl_2$	13.84	
14	0.09 gm. $MnCl_2$	15.44	14.64

On comparing these results with those in the ammonification tests, it is found that there was not perfect agreement. In the case of ammonification, the use of amounts of the chloride greater than 0.01 gm. or 200 pounds per acre depressed the process, but the nitrification tests seem to indicate that much larger amounts may be used with no danger of injurious effect on the process.

It seems that the results of the ammonification tests should be considered more conclusive than those of the nitrification experiments. In the first place, they were more definite, a fact which indicates that the method used is more suitable, while in the nitrification results, the variations in

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duplicate determinations were as large as differences between variously treated soils. This would suggest the possibility that the method for determining the nitrifying power of soils does not permit of perfect differentiation among soils differently treated. Perhaps a longer incubation period might have modified the results to the best extent, or some other slight change in the method may be advisable. The present results, however, must be interpreted as they stand.

It is customarily believed that the nitrifying organisms are more sensitive than the ammonifiers to an excess of salts in the soils, as well as to other unusual influences. This is due to the fact that the ammonifying group of organisms includes such a large number of bacteria of various characteristics and especially those with the ability to form spores. Furthermore, many influences which restrict the process of nitrification have been found to have little or no effect on ammonification.

Again, the fact that ammonification must precede nitrification, that the latter process can occur only following the former, precludes the possibility of the former's being depressed and the latter's being increased at the same time, at least in the case of normal soils. Under ordinary conditions ammonia does not accumulate in soils, and hence when a soil is subjected to treatment, unless ammonia production is increased, it is impossible for nitrate production to be increased. While, therefore, special treatments may restrict nitrification and not ammonification, in which case ammonia would accumulate in the soil, it is difficult to understand how the latter process could be depressed and not nitrification also.

In short, it appears that the conclusions from this work should be based on the ammonification tests and on the nitrification tests in so far as they are in agreement, and the apparent discrepancies in the latter results should be attributed to the method employed.

Manganese chloride, therefore, in amounts greater than 200 pounds per acre depressed ammonification, the depression increasing with the additions until a point was reached at which ammonification ceased. In amounts smaller than 200 pounds per acre, manganese chloride gave slight increases in ammonification, but these were not great enough to be conclusive, and the results should probably be interpreted as showing a lack of effect of the applications. With nitrification, amounts of the chloride greater than 2000 pounds per acre depressed the process, the depression increasing as in the case of ammonification until a point was reached where the process stopped. Smaller amounts of the manganese chloride, however, gave quite definite increases in nitrification, the application of 100 pounds giving the greatest effect.

It is probable, therefore, that the small increase noted in the ammonification results should be considered definite and the use of manganese chloride at the rate of 100 pounds per acre as bringing about an increase in the bacterial processes of nitrification and ammonification.

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If therefore, such an amount of the chloride applied to the soil increases plant growth, the effect may be attributed partly to the influence on ammonification and nitrification.

In amounts greater than 200 pounds per acre, the chloride depressed the activities of the ammonifiers and nitrifiers and an injurious action of a manganese fertilizer might, therefore, be due to its effect on these organisms.

Series V

The Effect of $MnSO_4$ on Ammonification

This series was planned to test the effect of manganese sulfate on ammonification. The applications made to the soil and the results secured are given in Table V.

TABLE V

THE EFFECT OF $MnSO_4$ ON AMMONIFICATION

No.	Treatment	Ammonia Mg. N.	Average Mg. N.
1	Check	265.28	
2	Check	262.83	264.06
3	0.1 gm. $MnSO_4$	256.90	
4	0.1 gm. $MnSO_4$	261.44	259.17
5	0.5 gm. $MnSO_4$	240.61	
6	0.5 gm. $MnSO_4$	258.41	249.51
7	1.0 gm. $MnSO_4$	218.39	
8	1.0 gm. $MnSO_4$	213.62	216.01

The use of the manganese sulfate seems to have depressed ammonification to a considerable extent, the larger amounts applied bringing about greater depressions. Only three applications of the sulfate were made here, 0.1 gm., 0.5 gm., and 1.0 gm., while in the case of the chloride, 3.0-gm. and 5.0-gm. amounts also were used, so that no complete comparisons can be made. It seems, however, that the sulfate did not bring about as pronounced a depression as the chloride did.

It is evident from this test that applications of manganese sulfate in amounts greater than 2000 pounds per acre depressed ammonification, and if any stimulation of the process is produced by the sulfate, it must be with a smaller amount than any used here.

Series VI

The Effect of $MnSO_4$ on Ammonification (Continued)

In order to test the effect on ammonification of smaller amounts of manganese sulfate than those used in the preceding series, a further test was arranged.

The amounts of sulfate added and the results secured appear in Table VI.

It is evident from an examination of this table that all the applications of the sulfate increased to some extent the ammonifying power of the

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soil. The greatest increase, however, occurred with the addition of 0.005 gm. With the 0.01, 0.03, 0.05, 0.07 and 0.09-gm. quantities almost identical amounts of ammonia were secured, the differences which were evidenced being attributable entirely to unavoidable variations in the determinations. The amounts produced, however, were less than that obtained where the 0.005-gm. quantity was used.

It appears, therefore, from these results that the application of 100 pounds of manganese sulfate per acre brought about a stimulation of ammonification which, while not large, was still quite definite. Increasing the application up to 2000 pounds per acre occasioned a smaller increase in ammonification but still a definite gain was evidenced. Beyond that point, however, the sulfate depressed the ammonifying power of the soil, the depression increasing with increasing applications.

TABLE VI

THE EFFECT OF $MnSO_4$ ON AMMONIFICATION—(Continued)

No.	Treatment	Ammonia Mg. N.	Average Mg. N.
1	Check	166.47	
2	Check	162.80	164.64
3	0.005 gm. $MnSO_4$	180.95	
4	0.005 gm. $MnSO_4$	188.48	184.72
5	0.01 gm. $MnSO_4$	168.44	
6	0.01 gm. $MnSO_4$	184.24	176.34
7	0.03 gm. $MnSO_4$	185.18	
8	0.03 gm. $MnSO_4$	164.59	174.89
9	0.05 gm. $MnSO_4$	169.10	
10	0.05 gm. $MnSO_4$	176.44	172.77
11	0.07 gm. $MnSO_4$	172.49	
12	0.07 gm. $MnSO_4$	184.90	178.70
13	0.09 gm. $MnSO_4$	174.27	
14	0.09 gm. $MnSO_4$	184.71	179.49

If manganese sulfate is applied to soils then in amounts smaller than 2000 pounds per acre, and exerts a beneficial effect on crop yields, the effect may be due, in part, at least to the acceleration of the ammonification process. If the use of 100 pounds per acre gives the best results, it may be due likewise to the fact that ammonification is accelerated to the greatest extent by that amount. On the other hand, if applications of 2000 pounds and over of manganese sulfate depress crop growth, it may be due, partly at any rate, to the depression in ammonification, indicating as it does a depression in the decomposition of organic matter and therefore in the production of available plant-food.

Series VII

The Effect of $MnSO_4$ on Nitrification

The effect of manganese sulfate on nitrification was tested in this series. The amounts of the sulfate used were the same as in the first ammonification test, Series V, except that two additional amounts, 3.0 gm. and 5.0 gm. were used.

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On turning to Table VII which gives the results of the tests, it is found that the sulfate depressed nitrification, the depression, with one exception, increasing with increasing additions of the salt. In one case, a larger amount of the sulfate seemed to give a smaller depression, but the difference was not great and no duplicate determination was secured. With the 3.0 and 5.0-gm. quantities, the same amounts of nitrates were secured, but the production was very small. Evidently these applications of the sulfate led to a minimum nitrification.

These results agreed quite satisfactorily with the ammonification results as far as they were comparable, the addition of 0.1 gm. depressing slightly both nitrification and ammonification, while the larger amounts gave large depressions, the extent of the depression depending on the size of the application.

TABLE VII

THE EFFECT OF $MnSO_4$ ON NITRIFICATION

No.	Treatment	Nitrate Mg. N.	Average Mg. N.
1	Check	22.38	
2	Check	30.22	26.30
3	0.1 gm. $MnSO_4$	21.06	
4	0.1 gm. $MnSO_4$	21.06	21.06
5	0.5 gm. $MnSO_4$	4.24	
6	0.5 gm. $MnSO_4$	6.64	5.44
7	1.0 gm. $MnSO_4$	6.64	
8	1.0 gm. $MnSO_4$	[1]12.96	6.64
9	3.0 gm. $MnSO_4$	2.76	
10	3.0 gm. $MnSO_4$	4.24	3.50
11	5.0 gm. $MnSO_4$	2.76	
12	5.0 gm. $MnSO_4$	4.24	3.50

[1] Omitted from the average.

Two thousand pounds of manganese sulfate, therefore, if applied to the soil may bring about a depression in crop growth because of a decrease in ammonification and in nitrification.

SERIES VIII

THE EFFECT OF $MnSO_4$ ON NITRIFICATION (CONTINUED)

The previous series showed the effect of manganese sulfate applied to the soil in amounts of 2000 pounds per acre and above. It remains to be determined whether smaller applications would accelerate nitrification and up to what point additions of the sulfate might be made with safety as regards its effect on nitrification.

The amounts of sulfate added and the results obtained are given in Table VIII. It is apparent from an examination of this table that the 0.005-gm. quantity of the sulfate brought about the greatest increase in nitrification. The amounts larger than this apparently had little effect, slight increases being noted, however, in most cases. The differences, however, were too slight to be conclusive, the variation between duplicate

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determinations being as great as that between the treated and check soils. It seems evident that 100 pounds of manganese sulfate per acre had the greatest effect on nitrification and larger quantities had less influence. A noticeable depression, however, did not occur until 2000 pounds per acre were used.

These results check very satisfactorily the ammonification results. In that case also, the 100-pound application brought about the greatest increase in bacterial action, and larger amounts depressed the action.

TABLE VIII

THE EFFECT OF $MnSO_4$ ON NITRIFICATION—(Continued)

No.	Treatment	Nitrate Mg. N.	Average Mg. N.
1	Check	15.22	
2	Check	16.02	15.62
3	0.005 gm. $MnSO_4$	16.90	
4	0.005 gm. $MnSO_4$	16.86	16.88
5	0.01 gm. $MnSO_4$	16.02	
6	0.01 gm. $MnSO_4$	15.22	15.62
7	0.03 gm. $MnSO_4$	15.22	
8	0.03 gm. $MnSO_4$	16.90	16.06
9	0.05 gm. $MnSO_4$	14.44	
10	0.05 gm. $MnSO_4$	16.02	15.23
11	0.07 gm. $MnSO_4$	16.00	
12	0.07 gm. $MnSO_4$	15.20	15.60
13	0.09 gm. $MnSO_4$	15.20	
14	0.09 gm. $MnSO_4$	16.00	15.60

These studies of the effects of manganese sulfate on ammonification and nitrification as a whole show quite distinctly that if the salt when applied to the soil at the rate of 100 pounds per acre increases the crop yield, the effect may be due in part at least to the increase in the ammonifying and nitrifying powers of the soil. With larger applications than that mentioned, up to 2000 pounds per acre, increases in plant growth may be due to gains in ammonification. The gains in nitrification were quite small or entirely absent. No depressions were observed, however. Whether gains in ammonification without similar increases in nitrification are of great importance is a moot question. There is considerable evidence in support of the belief that plants will use ammonium compounds as their nitrogen food, particularly if nitrates are lacking.

It seems probable, therefore, that when the ammonification process is increased and nitrification is not, plants may use the ammonium compounds which are formed and increased plant growth may result.

When the application of manganese sulfate in amounts greater than 2000 pounds per acre is made, depressions in crop yields would be due in part to decreases in ammonification and in nitrification and consequent deficiencies in plant-food available for the crop grown.

Manganese sulfate and manganese chloride seem to act very similarly on ammonification and on nitrification. In amounts greater than 100

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pounds per acre depressions in both processes occurred, while when applied at the rate of 100 pounds per acre more or less definite gains in the activities of both groups of organisms were found.

SERIES IX

THE EFFECT OF $Mn(NO_3)_2$ ON AMMONIFICATION

Manganese nitrate was the next salt tested for its effect on ammonification and on nitrification and this series was arranged to show the effect on the former process.

Table IX contains the results secured and also shows the amounts of the nitrate applied. Only three quantities were used here, just as in the case of the sulfate tests, but the depression in ammonification was so definitely shown that greater amounts were unnecessary. With the addition of 0.1 gm. of the nitrate, a slight gain in ammonification was given, but the duplicate results did not check exactly and if the low result instead of the average of the low and the high were chosen, a slight depression in the ammonifying power of the soil would be shown. It is apparent, therefore, that this result should not be considered definite and the subsequent ammonification tests and the nitrification results may indicate which interpretation should be put on these results.

TABLE IX

THE EFFECT OF $Mn(NO_3)_2$ ON AMMONIFICATION

No.	Treatment	Ammonia Mg. N.	Average Mg. N.
1	Check	265.28	
2	Check	262.83	264.06
3	[1]0.1 gm. $Mn(NO_3)_2$	278.19	
4	0.1 gm. $Mn(NO_3)_2$	259.81	269.00
5	0.5 gm. $Mn(NO_3)_2$	193.49	
6	0.5 gm. $Mn(NO_3)_2$	169.87	181.68
7	1.0 gm. $Mn(NO_3)_2$	132.29	
8	1.0 gm. $Mn(NO_3)_2$	129.85	131.07

[1] Application made on the water-free basis.

With the larger applications definite depressions were brought about, the depression increasing with the size of the application.

SERIES X

THE EFFECT OF $Mn(NO_3)_2$ ON AMMONIFICATION (CONTINUED)

In order to test the effect of smaller amounts of manganese nitrate than those used in the preceeding test, on ammonification, this series was arranged. The amounts used were different in some cases from those of the chloride and sulfate used in the preceeding series, due to preliminary observations from the previous series, but comparisons are, nevertheless, possible in several instances.

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On examining Table X, which gives the results, it appears that the smallest amount of the nitrate, 0.025 gm. gave a slight increase in ammonification but the results were again not definite, the duplicate determinations not agreeing satisfactorily. If the low result be chosen as the most accurate, a depression from the nitrate appeared while the average results showed a gain in ammonification. The nitrification results may throw some light on this point, but the present result was inconclusive.

With larger applications of the manganese nitrate, however, definite depressions in ammonification were given, the depression increasing with increasing additions, up to 0.50 gm. It is evident, therefore, that in the previous series the use of 0.1 gm. of the nitrate must have given a depression in ammonification, that is, the low determination should have been considered, rather than the average of the duplicates, which did not agree as has been mentioned. The amounts of ammonia produced here were not so great and the differences appeared much more distinctly largely on that account.

TABLE X

THE EFFECT OF $Mn(NO_3)_2$ ON AMMONIFICATION—(Continued)

No.	Treatment	Ammonia Mg. N.	Average Mg. N.
1	Check	166.96	
2	Check	169.68	168.32
3	[1]0.025 gm. $Mn(NO_3)_2$	161.14	
4	0.025 gm. $Mn(NO_3)_2$	188.63	174.89
5	0.05 gm. $Mn(NO_3)_2$	166.03	
6	0.05 gm. $Mn(NO_3)_2$	[2]149.09	166.03
7	0.10 gm. $Mn(NO_3)_2$	168.46	
8	0.10 gm. $Mn(NO_3)_2$	157.14	162.80
9	0.20 gm. $Mn(NO_3)_2$	146.52	
10	0.20 gm. $Mn(NO_3)_2$	147.83	147.18
11	0.35 gm. $Mn(NO_3)_2$	124.57	
12	0.35 gm. $Mn(NO_3)_2$	117.81	121.19
13	0.50 gm. $Mn(NO_3)_2$	109.28	
14	0.50 gm. $Mn(NO_3)_2$	103.37	106.33

[1] Applications made on the water-free basis. [2] Omitted from the average.

The use of manganese nitrate on this soil apparently depressed ammonification even when used in small amounts. There was some question about the effect of the use of the nitrate at the rate of 500 pounds per acre but with larger applications the depressions were very definite. The nitrification results may indicate whether a depression from the 500-pound application should be accepted as the case or whether a slight increase should be concluded as occurring.

If, therefore, manganese nitrate is applied to the soil at the rate of 500 pounds per acre and an increase in crop yield is secured, this is probably not brought about by an effect on ammonification. If that or larger amounts of the salt when added to the soil depress crop growth, that depression may be partly at least a result of decreased ammonification.

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SERIES XI

THE EFFECT OF $Mn(NO_3)_2$ ON NITRIFICATION

This series was arranged to test the effect of manganese nitrate on nitrification. The results of the test as well as the arrangement appear in Table XI. It was necessary, of course, to calculate the nitrate recovered from the ammonium sulfate nitrified, and subtract the nitrate added as manganese nitrate in order to ascertain the actual nitrification occurring in the soil. The manganese nitrate was added in solution in amounts sufficient to make an application of water-free nitrate in the amounts given.

On examining the results in the table, it appears that all the applications of manganese nitrate depressed the nitrifying power of the soil, for in no case was the entire amount of nitrate added as the manganese nitrate recovered. The lack of recovery of the nitrate added may be interpreted as showing the depression in nitrification. In other words, when 4.32 mg. of nitrogen as nitrate is given as the amount of nitrate not recovered, that amount represents the actual depression in nitrification expressed in milligrams of nitrogen.

TABLE XI

THE EFFECT OF $Mn(NO_3)_2$ ON NITRIFICATION

No.	Treatment	Nitrate Mg. N.	Average Mg. N.	Mg. N. as NO_3 recovered	Mg. N. as NO_3 added	Mg. N. not recovered
1	Check	16.87				
2	Check	12.66	14.76	.....	.....	.....
3	[1]0.1 gm. $Mn(NO_3)_2$	28.12				
4	0.1 gm. $Mn(NO_3)_2$	24.11	26.11	11.35	15.66	4.32
5	0.5 gm. $Mn(NO_3)_2$	94.92				
6	0.5 gm. $Mn(NO_3)_2$	74.92	85.42	70.66	78.30	7.64
7	1.0 gm. $Mn(NO_3)_2$	94.92				
8	1.0 gm. $Mn(NO_3)_2$	98.88	96.90	82.14	156.60	74.46

[1] Applications made on the water-free basis.

It is evident, therefore, that the larger amounts of the manganese nitrate caused greater depressions in nitrification than the smaller amount. Of course, there was probably some assimilation of the nitrate added and it is impossible to ascertain the relative importance of assimilation and nitrification. The result obtained with the addition of 1.0 gm. of the nitrate indicates that assimilation did not occur to a very great extent, for the lack of recovery was almost equal to the total nitrification. If it did play a part here, its importance was probably not great.

These results show that the application of manganese nitrate at the rate of 2000 pounds per acre depressed nitrification and larger applications brought about greater depressions.

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The ammonification results were checked by these for nitrification and it would seem that the doubt in the former results regarding the effect of the smallest application should be removed and a slight depression should be considered as occurring.

Series XII

The Effect of $Mn(NO_3)_2$ on Nitrification (Continued)

This series is a continuation of the preceeding, the amounts of manganese nitrate used being smaller than those employed in that case. The same quantities as were used in Series X in the ammonification tests were employed here.

TABLE XII

THE EFFECT OF $Mn(NO_3)_2$ ON NITRIFICATION—(Continued)

No.	Treatment	Nitrate Mg. N.	Average Mg. N.	Mg. N. as NO_3 recovered	Mg. N. as NO_3 added	Mg. N. not recovered
1	Check	22.22				
2	Check	20.78	21.50	.....	.....	.....
3	[1]0.025 gm. $Mn(NO_3)_2$	22.88				
4	0.025 gm. $Mn(NO_3)_2$	22.22	22.50	1.00	3.92	2.92
5	0.05 gm. $Mn(NO_3)_2$	19.70				
6	0.05 gm. $Mn(NO_3)_2$	20.00	19.85	—1.65	7.83	9.48
7	0.10 gm. $Mn(NO_3)_2$	24.68				
8	0.10 gm. $Mn(NO_3)_2$	26.66	25.67	4.17	15.66	11.49
9	0.20 gm. $Mn(NO_3)_2$	28.88				
10	0.20 gm. $Mn(NO_3)_2$	40.64	34.76	13.26	31.32	18.06
11	0.35 gm. $Mn(NO_3)_2$	[2]83.00				
12	0.35 gm. $Mn(NO_3)_2$	53.96	53.96	32.46	54.81	22.35
13	0.50 gm. $Mn(NO_3)_2$	60.72				
14	0.50 gm. $Mn(NO_3)_2$	53.96	57.34	35.84	78.30	42.56

[1] Applications made on the water-free basis. [2] Omitted from the average.

Turning to Table XII which gives the results as well as the arrangement of the test, it is apparent that the smallest amount of the nitrate, 0.025 gm., brought about a depression in nitrification. The larger amounts of the salt brought about greater depressions the extent of the depression varying directly with the size of the application. The results obtained in the previous series were thus checked by those secured here. The application of manganese nitrate even in small amounts depressed nitrification to a considerable extent. The smallest amount used here was 500 pounds per acre. Larger applications depressed the nitrifying action still more extensively, in some cases very little nitrification having occurred.

Assimilation may have played a part in these experiments also but how extensive it may have been under the particular experimental conditions is difficult to ascertain.

These nitrification results served to check and emphasize the ammonification results. The depression here with the smallest application of the

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nitrate led to the conclusion that the depression in the case of the ammonification results was the action which should be accepted as accurate. The results with the larger amounts of the nitrate compared quite completely with those where the ammonifying power was tested. Depressions increasing with increasing applications were noted in both cases.

It is evident from these results that manganese nitrate in amounts of 500 pounds per acre and above depressed ammonification and nitrification, the depression increasing with increasing applications. If this salt of manganese in any of these amounts gives a favorable effect on plant growth, it must be due to its influence on some other factors than these two tested here. It may be due to influence on some other bacteria, or to oxidative or catalytic influences as has been suggested. If other groups of bacteria are influenced they may be particular groups concerned in the destruction of some special substances.

Of course, the amounts of manganese nitrate used were larger than those of the chloride and the sulfate which were found to increase the processes of ammonification and nitrification to some extent. If smaller amounts of the nitrate had been used, perhaps increases might have occurred here. It is apparent, however, that application of 500 pounds and over depressed the transformation of nitrogen compounds and hence if such amounts were found to depress crop yields, it might be due to their restricting influence on ammonification and nitrification processes, hence restricting the production of available plant-food.

Series XIII

The Effect of MnO on Ammonification

The influence of manganous oxide on ammonification was tested in this series. The amounts of the oxide employed were the same as those used in the case of the other salts tested. The results of the tests appear in Table XIII.

On examining this table it is apparent that all the applications depressed the ammonifying power of the soil. The depression with the 0.1 gm. of MnO was very pronounced but the larger applications did not increase the depression. The 5-gm. quantity of the oxide depressed the process about the same as the 0.1-gm. quantity. Some variations in the results were found, due to non-agreement of duplicate determinations and hence it was impossible to draw definite conclusions regarding the relative effect of the various applications. It is clearly evident, however, that the oxide in all amounts used depressed considerably the ammonifying power of the soil.

If manganous oxide is applied to the soil at the rate of 2000 pounds or over and shows a beneficial effect, it must be due to some other influence than that on ammonification. If, on the other hand, the application shows a depression, the depressing effect on ammonification might account for the effect.

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Smaller amounts of the manganous oxide were not tested so that it is impossible to compare the results with this compound with those secured with the other manganese salts.

TABLE XIII

THE EFFECT OF MnO ON AMMONIFICATION

No.	Treatment	Ammonia Mg. N.	Average Mg. N.
1	Check	463.18	
2	Check	473.10	468.14
3	0.1 gm. MnO	441.40	
4	0.1 gm. MnO	457.94	449.67
5	0.5 gm. NnO	467.04	
6	0.5 gm. MnO	447.74	457.39
7	1.0 gm. MnO	466.48	
8	1.0 gm. MnO	421.25	443.87
9	3.0 gm. MnO	437.26	
10	3.0 gm. MnO	444.65	440.96
11	5.0 gm. MnO	443.33	
12	5.0 gm. MnO	452.70	448.02

Smaller amounts may have brought about slight increases as they did in the case of manganese chloride and manganese sulfate, but this may only be considered a possibility.

SERIES XIV

THE EFFECT OF MnO ON NITRIFICATION

In order to test the effect of manganous oxide on nitrification, this series was arranged. The amounts of the oxide applied were the same as those used in the preceeding series.

The arrangement of the tests and the results are given in Table XIV.

TABLE XIV

THE EFFECT OF MnO ON NITRIFICATION

No.	Treatment	Nitrate Mg. N.	Average Mg. N.
1	Check	27.77	
2	Check	20.83	24.30
3	0.1 gm. MnO	8.00	
4	0.1 gm. MnO	4.00	6.00
5	0.5 gm. MnO	2.96	
6	0.5 gm. MnO	7.11	5.04
7	1.0 gm. MnO	2.50	
8	1.0 gm. MnO	8.00	5.25
9	3.0 gm. MnO	20.00	
10	3.0 gm. MnO	16.00	18.00
11	5.0 gm. MnO	.2.00	
12	5.0 gm. MnO	2.47	2.24

On examining the table, it becomes evident that all the applications of manganous oxide depressed nitrification. With one exception the depressions were very large. In the case of the 3.0-gm. addition, only a very slight depression was found, but inasmuch as the applications of 1.0 gm. and of 5.0 gm. both depressed the process to a large extent, it seems prob-

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able that this result was due to some error in arranging the series or perhaps to some accidental factor interfering in the results. It appears, therefore, that the conclusion would be justified that manganous oxide depressed nitrification in all applications, the greatest depression occurring with the largest application.

These results confirm the previous ammonification tests and show that manganous oxide when applied to the soil in amounts equal to or greater than 2000 pounds per acre depressed both ammonification and nitrification, the depression increasing slightly with the amount of the oxide applied, but almost as large a depression occurred with the use of 2000 pound per acre as with larger amounts.

If manganous oxide, when applied to the soil, depresses crop growth it may evidently be due to a decrease in the bacterial activities involved in the processes of ammonification and nitrification.

Smaller amounts of manganous oxide might increase bacterial action but this point was not tested in this work.

Conclusions

These experiments on the effect of certain manganese salts on ammonification and nitrification lead, therefore, to the following conclusions:

1. Manganese chloride in applications greater than 2000 pounds per acre depressed both ammonification and nitrification, the depression increasing as the size of the application was increased, until a point was reached at which both processes ceased.

2. With smaller amounts of the chloride the effects on the two processes were not identical but tended in the same direction. Thus the applications of 100 and 200 pounds per acre gave increases which were slight in the case of ammonification but quite distinct in the case of nitrification. With amounts greater than 200 pounds per acre and less than 2000 pounds however, ammonification was depressed while no appreciable depression was apparent on nitrification.

3. Manganese sulfate when applied to the soil at the rate of 100 pounds per acre increased appreciably both ammonification and nitrification.

4. In amounts greater than 100 pounds per acre and less than 2000 pounds, ammonification was increased but to a smaller extent than with the 100-pound application, but with nitrification no gains or depressions were found with these amounts.

5. In applications equal to or greater than 2000 pounds per acre, both nitrification and ammonification were depressed by manganese sulfate, the depression increasing with the size of the application.

6. Manganese nitrate added to the soil at the rate of 500 pounds per acre or in greater amounts depressed both ammonification and nitrifica-

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tion, the depression increasing as the size of the application was increased.

7. Manganous oxide when applied to the soil at the rate of 2000 pounds per acre or in larger quantities depressed both ammonification and nitrification, the depression becoming greater as the size of the addition was increased.

8. If manganese salts in small quantities increase crop yields on a soil, that increase may be due in part at least to a beneficial effect on ammonification and nitrification with a consequently greater production of avaliable plant-food.

9. On the other hand, if manganese salts when applied to the soil restrict crop growth, that restriction may be due in part to a depression of bacterial activity.

10. The amounts of various manganese salts which may be applied to any one soil without danger of depressing ammonification and nitrification are exceedingly variable.

Literature Cited

(1) Hillebrand, W. F.
1910. The analysis of silicate and carbonate rocks. U. S. Geol. Survey Bul. 422, 239 p.

(2) Kelley, W. P.
1912. The origin, composition and properties of the manganiferous soils of Oahu. *In* Hawaii Agr. Exp. Sta. Bul. 26, p. 54.

(3) Kelley, W. P.
1915. Ammonification and nitrification in Hawaiian soils. Hawaii Agr. Exp. Sta. Bul. 37, p. 45.

(5) Montanari, C.
1914. The influence of manganese oxides on nitrification. *In* Staz. Sper. Agr. Ital., v. 47, p. 817-852. Ref. *in* Chem. Abs. v. 9, no. 22, p. 3113.

(5) Montancira, C.
1914. The action of catalytic elements on nitrifying bacteria. *In* Staz. Sper. Ital., v. 47, p. 441-448. Ref. *in* Chem. Abs., v. 9, no. 6, p. 837.

(6) Skinner, J. J., Sullivan, M. X., et al.
1914. The action of manganese in soils. U. S. Dept. Agr. Bul. 42, 32 p.

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PRELIMINARY INVESTIGATIONS IN COMPARISON OF FIELD WITH LABORATORY EXPERIMENTS IN SOIL BIOLOGY

By

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The ultimate aim of any phase of scientific research is always its practical application. In many lines of investigation, however, methods of procedure have not yet been perfected to the extent that definite conclusions can be drawn from the results obtained.

To get the most accurate and complete results of all the factors which play a part in agricultural experimentation, the processes are first pursued on large areas in the field, then in specialized plots, then in tanks which are exposed to the out-door agencies. Further, in order to control the moisture and temperature conditions to a better advantage, vegetation experiments are carried on in the greenhouse. To combine all of these processes and in considering the ultimate constituents, the experimentation is finally completed in the laboratory.

Inasmuch as crop and fertilizer experiments are usually carried out in the field, the writer, in order to collect some data as to the influence of out-door conditions upon problems in soil biology, undertook to perform a preliminary series of experiments dealing with this subject.

A small plot 30 feet long and 9 feet wide was selected in a plowed field of the College Farm. The soil of this field was of a loam type, very nearly approaching Penn loam. A similar plot was taken in the orchard. This soil was of a Silt loam type. A third plot of the same size was staked off on a sandy soil. Hereafter, through this discussion, these soils will be designated as shale, orchard, and plot, respectively.

Each of these plots was carefully divided into 33 small areas as shown in figure 1.

These small areas, which were 6 inches square, were 18 inches apart. It was found that a section of soil 6 x 6 x 4 inches weighed 6 pounds. In each plot the small areas numbering 1 to 12 represent the ammonification series, 13 to 24 inclusive represent the areas used for the nitrification experiments, and 25 to 33 were the areas plotted off for nitrogen fixation. Area No. 1 received no treatment and the soil was not disturbed, No. 2 received dried blood, No. 3 was dug out and was put through the same

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process as in the case where additions were made. Area No. 4 received cottonseed meal. Then areas Nos. 5, 6, 7 and 8 correspond with 1, 2, 3 and 4, respectively. Likewise do areas Nos. 9, 10, 11 and 12. No. 13 was a blank with no treatment, as was No. 1. No. 14 received ammonium sulphate, No. 15 was a blank as in the case of No. 3, No. 16 received dried blood, then Nos. 17, 18, 19 and 20 and Nos. 21, 22, 23 and 24 corresponded with 13, 14, 15 and 16, respectively. No. 25 was a blank with no treatment as in the case of No. 1. No. 26 received dextrose. No. 27 received no application and got the same treatment as No. 3, then Nos. 28, 29 and 30 and 31, 32 and 33 were treated as 25, 26 and 27, respectively.

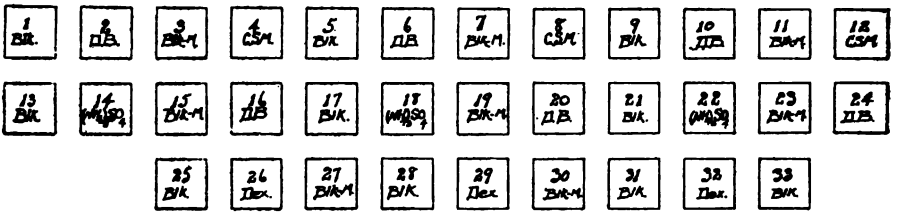

Fig. 1.—Diagram of plot containing 33 areas used in comparing field and laboratory experiments in soil biology. Areas are 6 inches square and 4 inches deep, and are placed 18 inches apart.
Blk.—Blank, unmixed; Blk. M.—Blank, mixed; D. B.—Dried blood, C. S. M.—Cottonseed meal; $(NH_4)_2\ SO_4$—Ammonium Sulfate; Dex.—Dextrose.

On July 30, 1915, a series of ammonification experiments, comparing field tests with those in the laboratory were begun. By means of a hatchet, a section of soil 6 x 6 x 4 inches, weighing 6 pounds, was carefully taken out, and enough dried blood or cottonseed meal carefully mixed with it to make the proportion of 155 mg. of nitrogen for every 100 gm. of soil. In the field there were three areas for each treatment, i. e., the experiment was carried out in triplicate on each soil. Likewise, there were three blank areas which had been dug up and thoroughly "mixed" but to which nothing had been added. In all cases where the soil of the small areas was dug up, it was replaced in its original position after the mixing had been accomplished. It was then compacted so that the surface of the area was level with the surrounding soil.

At the time that the organic matter was added to the field areas, a large sample of each of these soils was taken to the laboratory and the percentage of moisture was determined by the official method. Three 100-gm. portions of each soil were carefully weighed out. To these portions enough dried blood was added so that there were 155 mg. of nitrogen to each portion of soil. Likewise, into three 100-gm. portions of each soil, cottonseed meal equivalent to 155 mg. of nitrogen was mixed;

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and a third series of three portions to which no organic matter was added was also weighed out. These portions of soil were placed in tumblers and incubated for a period of 7 days at 24° to 25° C.; organic matter added to the field also had been allowed to ammonify for a period of 7 days. At the expiration of 7 days, a composite sample of 200 gm. was brought to the laboratory. Moisture determinations of the soil of each were made. One-hundred-gram portions of these field soils were then weighed out into copper flasks and the ammonia formed from the decomposition of the organic matter was distilled off by the addition of about 10 gm. of magnesium oxide. At the same time, the ammonia formed in the samples incubated in the laboratory was distilled off in like manner. The results were calculated on the oven-dry basis.

In order to get some data as to the nitrification of nitrogenous materials in the field relative with that under controlled conditions in the laboratory, ammonium sulfate and dried blood were added to small field areas, as was the cottonseed meal and dried blood in the ammonification experiments. Ammonium sulfate to the extent of 100 mg. for every 100 gm. of soil was mixed with the soils of the field plots. On the areas used to demonstrate the nitrification of dried blood, for each 100 gm. of soil a quantity of dried blood equivalent to the nitrogen in 100 mg. of ammonium sulfate was added. The nitrification experiments were also duplicated in tumblers in the laboratory. The soils were allowed to incubate for a period of 33 days, after which time the amounts of nitrates formed were determined by the official colorimetric methods.

To a third series of field areas used to demonstrate the nitrogen fixation of the soils, dextrose to the extent of 2 gm. per 100 gm. of soil was added to each 6-pound portion. After a period of 10 days' incubation, the soils were air-dried and the nitrogen determinations made. Samples of 10 gm. each were used and were titrated with N/20 acid. The other details of sampling and treatment of the field experiments, as well as the laboratory samples, were carried out as in the case of the ammonification studies.

It is seen from Table I, that while ammonification takes place quite rapidly in the field, the organic matter does not decay as rapidly as it does in the laboratory. In only one case there was more organic matter decomposed in the field than in the laboratory tests. That there was greater ammonification in the laboratory experiments than in the field might be attributed to several causes, one of which was the moisture factor due to excessive rainfall. No doubt great variation in the temperature of the soil in the field had a considerable effect upon the process. During the night, the temperature of the soil was lowered so that the rate of decay was greatly reduced, while in the laboratory, where the temperature was constant, these processes could go on without being

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TABLE I

AMMONIFICATION OF DRIED BLOOD AND COTTONSEED MEAL BY THREE TYPES OF SOIL UNDER CONTROLLED CONDITIONS IN THE LABORATORY COMPARED WITH THEIR AMMONIFICATION IN THE FIELD

	Determinations in the Laboratory							Determinations incubated in the Field						
Kind of Soil	Blank Determinations mg. N.	Average mg. N.	Dried Blood mg. N.	Average minus Blank. mg. N.	Cottonseed Meal mg. N.	Average minus Blank. mg. N.	Blank Soil Not stirred mg. N.	Determinations Soil stirred mg. N.	Dried Blood mg. N.	Dried Blood minus Blank mg. N.	Dried Blood Average mg. N.	Cottonseed Meal mg. N	Cottonseed Meal minus Blank mg. N.	Cottonseed Meal Average mg. N.
Shale	2.05		26.50		68.15		1.96	4.65	42.20	37.55		64.70	60.05	
Shale	1.56		30.80		68.50		2.32	3.27	44.60	41.33		57.10	53.83	
Shale	1.38	1.66	28.53	26.95	68.20	66.62	1.34	1.64	46.15	44.51	41.13	62.40	60.76	58.21
Orchard	1.92		38.10		76.20		1.61	2.66	29.25	26.59		57.28	54.62	
Orchard	1.55		37.13		73.00		1.70	1.57	28.37	26.80		58.80	57.23	
Orchard	1.38	1.62	37.10	35.82	74.95	73.10	1.78	1.46	32.12	30.66	28.01	65.78	64.32	58.72
Plot	2.46		34.80		80.78		1.06	2.34	28.20	25.86		56.75	54.41	
Plot	Lost		33.42		80.60		1.34	1.43	29.62	28.19		66.00	64.57	
Plot	2.03	2.24	35.05	32.18	78.46	77.70	1.51	1.34	23.22	21.88	25.31	66.82	65.48	61.48

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influenced. Lack of aeration in the field areas is another important factor which must be considered. A third feature of importance is the excess of moisture in the field samples as is seen by the rainfall during the time that these soils were incubated.

With all the soils, in both the field and the laboratory experiments, there was a greater amount of cottonseed meal ammonified than dried blood. With one exception, twice as much cottonseed meal as dried blood was decomposed. There seemed to be very little difference in the ammonifying power of the different soils. That stirring the soil affects biological activities to a marked extent is apparent when one compares the relative amounts of ammonia distilled from the small areas which had been stirred with those which remained undisturbed.

TABLE II

THE VARIATION IN NITROGEN CONTENT OF SMALL AREAS 6 x 6 INCHES AND 18 INCHES APART

Area No.	Kind of Soil		
	Shale gm. N. in 100 gm. soil	Orchard gm. N. in 100 gm. soil	Plot gm. N. in 100 gm. soil
25	.1846	.1344	.1138
26	.1846	.1428	.1078
27	.1867	.1380	.1135
28	.1827	.1318	.1003
29	.1850	.1367	.1007
30	.1867	.1349	.0999
31	.1993	.1249	.0979
32	.2098	.1120	.0927
33	.2074	.1108	.0958

Inasmuch as there was an excessive amount of rainfall during the time that the nitrification and nitrogen fixation experiments were carried on, the field experiments with regard to moisture particularly were under conditions very different from those affecting the corresponding tests in the laboratory. The lack of nitrates in the field areas, to which ammonium sulphate had been added, as in several instances none was found, clearly demonstrated that the heavy rains either washed it away as such or as nitrates after it had been nitrified. Likewise, the nitrates resulting from the nitrification of the dried blood must have either been leached away very soon after they had been formed, or fixed at lower depths. No doubt there occurred a solution and loss of the dextrose in the nitrogen fixation experiments which were carried out in the field, as there was a heavy fall of rain during the ten days that this experiment was under observation. Due to these very abnormal conditions of moisture, the results obtained in the nitrification and nitrogen fixation tests in the field are not entered in this preliminary paper, as a comparison between field and laboratory test of these processes under such conditions would hardly be justified.

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The data in Table II show an interesting fact in regard to the variation in the nitrogen content over a small area of a field, which was made plain during this experiment.

It is seen in Table II, that the nitrogen content of the extreme areas, Nos. 25 and 33, which were 15½ feet apart, in the case of the plot soil varied as much as .0180 gm. while with the orchard soil this difference was .0236 gm. Thus it is very apparent that great care must be exercised in sampling a field if a uniform sample is desired.

From the limited amount of data presented, it appears that, to some extent at least, biological experiments can be carried out in the field. Inasmuch as the moisture is one of the controlling factors, to be most successful with such experiments, a season during the year must be selected when there is apt to be least danger of heavy rainfall.

Summary

1. Biological experiments (e. g. in ammonification) can be successfully carried out in the field.

2. As a rule a greater amount of organic matter seems to be ammonified in the laboratory tests than in the field.

3. Nitrogen fixation and nitrification studies in the field are greatly interfered with by rains.

4. The nitrogen content of the soil varies considerably, even over a comparatively small area.

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A STUDY OF THE ACTION OF CARBON BLACK AND SIMILAR ABSORBING MATERIALS IN SOILS[1]

By

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Carbon black is a very good agent for purifying distilled water for plant physiological purposes, its action seeming to be one of absorbing substances of a solid or gaseous nature. It is used quite generally by plant physiologists in preparing good water for culture work. Other insoluble, finally divided materials, such as ferric hydrate, aluminum hydrate, magnesium carbonate, barium carbonate and quartz flour (1, 3) are good purifiers of distilled water and extracts of soils.

Water extracts of certain unproductive soils are improved by shaking them with carbon or ferric hydrate and filtering the solution clear (2, 3). Experiments were made to ascertain whether certain poor soils would be improved by the addition of carbon and other finely divided materials direct to the soil. These experiments were made first in pots in the greenhouse, using soils which had grown the same crop repeatedly for a number of times and had become very poor. The productivity of these soils was not restored by fertilizers. Carbon black was added to and mixed with the soil in an attempt to absorb anything of a harmful nature from the soil, but the soil was not thereby improved for plant growth. In a field experiment on the Arlington Experimental Farm carbon black was added on plots growing wheat, rye, timothy, clover, corn, cowpeas, and potatoes. This experiment was conducted for six years, the same crop having been grown on the same plot each year. The carbon had no beneficial effects on any of the plots. These experiments were made by mixing the carbon with the soil. Although the carbon might have had an absorptive action in taking up substances of a harmful nature, it was nevertheless in close contact with the soil and plant roots. On this account it might be expected that no beneficial action would be shown. Similar experiments were made, in which ferric hydrate and magnesium carbonate were used, but no uniformly beneficial results were secured.

Experiments were made in similar soils by putting the carbon in porous pots, tubes, and jars and burying them in the soil. The tubes used were very porous, permitting the moisture of the soil to pass freely through the

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carbon and out again. Through the use of this method the absorbing material was not brought in contact with the soil itself or with the plant roots, but was able to absorb materials from the soil solution. If the soil contained soluble, harmful organic substances they would to a certain extent be absorbed and removed from the solution. Experiments bearing on this problem were made and are presented in the following pages. The carbon black used throughout this investigation is made by burning natural gas and collecting the carbon on cooled cylinders. It is known as the "G Elf" brand and was secured from G. L. Cabot and Co., Boston, Mass.

Effect of Carbon Black Incased in Porous Material on Growth in Pots

Several experiments were made in pots, carbon black being placed in a smaller pot of porous earthenware material and then buried in the soil in the larger pot in which the plants grew. The carbon black used in these experiments was thoroughly washed and packed in the small pot in a moist condition. The pots were the ordinary unglazed flower pots used in general greenhouse work. On account of the porous character of the pots water added to the soil during the experiment could circulate easily through the soil into the incased carbon and back again through the walls of the inner pot into the soil in which the plants were growing. The roots of the plants could in no way come in contact with the carbon black, as the top of the inner pot was covered in such a way that the soil could not mix with the carbon.

A soil was used for this first experiment which had grown cowpeas in the greenhouse, crop after crop for two years. The soil had become very poor and produced very poor cowpeas. The soil was potted in 8-inch earthenware pots and a 4-inch pot was filled with carbon and buried in the center of the 8-inch pot. In the other pot, which was to serve as a check, the small inner pot was filled with some of the same soil as the larger pot. Nine cowpea plants were planted around the circumference of the pots 1 inch from the walls. The seeds were planted March 10, 1910, and grew for 6 weeks. The growth in the two pots at an early period of the experiment is shown in Plate I, (fig. 1). Pot No. 1 is the check and No. 2 contains the carbon black. It is shown here that the growth at this early stage is better in the pot containing carbon. The green weight of the nine plants at the end of six weeks for the carbon pot was 17.8 gm. against 13.9 gm. for the check plot, an increase of 29 per cent.

A similar experiment was made growing wheat in a sandy loam soil. This soil when used in the greenhouse in pots and boxes grew good wheat. Pots of the same size as those described under the preceding experiment were used. Ten wheat plants grew in each pot and were planted around the circumference as in the case of the cowpeas. The wheat was planted

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March 10 and the green weights taken April 11. The growth in the two pots was practically the same. The check pot produced 10.3 gm. green weight, while the green weight of the plants in the carbon pot was 10.4 gm.

Another experiment was made with wheat. This time a poor silty clay loam from the Arlington Experimental Farm was used. Eight-inch pots were used and the carbon, as before, was incased in a 4-inch pot and buried in the soil of the larger pot. Ten wheat plants were grown in each pot for 4 weeks. The green weight of the plants in the check pot was 3.9 gm. and that of the pots containing carbon was 6.6 gm., an increase of 70 per cent.

Still another test of this nature was made. The soil used was taken from the Smithsonian grounds and was so situated that it received the drainage and dripping from maple trees. Lawn grass in this section of the park invariably fails. Manure, lime, and commercial fertilizers have been used in attempts to secure a lawn, but with the same result, a complete failure. This ground was annually dug up and re-seeded in the early spring for several years, but the grass always failed.

The lawn soil was used in 8-inch pots as before. In one of these pots carbon incased in a small earthenware pot was buried, and in another, which was to serve as a check, the small buried pot was filled merely with the same soil used in the experiment. A mixed lawn grass seed was sown, the same amount in each pot. The grass was seeded April 12 and was cut for the first time May 11. A second cutting was made June 6, and a third June 28. The green weights of the grass are given in Table I.

TABLE I

EFFECT OF CARBON BLACK ON THE GROWTH OF GRASS IN A POOR LAWN SOIL

	First cutting gm.	Second cutting gm.	Third cutting gm.
Check	6.7	8.0	5.5
Carbon	10.6	10.0	9.0

The figures in the table show that the carbon had a very beneficial effect in this soil.

The same soil was used in a similar experiment in which clover was grown instead of grass. The check pot and carbon pot growing clover are shown in Plate I (fig. 2). The clover was planted April 12 and cut and weighed June 14. The green weight of the check pot was 90.0 gm. and that of the carbon pot 105.5 gm.

The beneficial action of carbon incased in porous material in these poor soils can be attributed only to its absorbing qualities. It would seem that the soil moisture, passing through the carbon, is robbed of its harm-

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ful material, whether organic or inorganic, gaseous or liquid, and the purified soil solution passing again into the soil becomes a better medium for the growth of plants.

Effects of Carbon Black in Porous Battery Jars in Soil in Greenhouse Benches

The principle of the adsorption of harmful organic material by carbon black from poor soil was tried on a larger scale with soil on greenhouse benches. The bench used is 3 feet 6 inches wide and 8 inches deep. Partitions were placed in the bench 18 inches apart. This makes a frame 36 inches by 18 inches by 8 inches and holds approximately 250 pounds of soil. In this type of experiment, battery jars of very porous material were used. The jars are 6 inches long and 2¾ inches in diameter. They were filled and well packed with moist washed carbon, corked and buried in the soil. The jars were laid in the bed in two parallel rows, five to each row. Each row of jars was approximately 6 inches from the side of the frame and the rows were 6 inches apart. They were covered with approximately 4 inches of soil. One frame contained the jars filled with carbon. To serve as a check, the adjoining frame contained the same number of jars filled with some of the soil used in the beds.

The soil used in this experiment was taken from the flower gardens of Mount Vernon, Virginia. This soil has been under investigation by this office for several years. Some parts of the garden are producing unsatisfactory growth in spite of the fact that the soil has been well manured. Salicylic aldehyde and several other organic compounds were found in soil from certain sections of the Mount Vernon garden in former investigations and this particular sample taken for the present investigation, when subjected to the chemical process for isolating aldehydes, revealed a substance which gave the aldehyde reactions with certain chemicals, showing the presence of this class of substances in the soil (4, 5).

String beans were planted in the soil in the soil fertility greenhouse at Arlington, Va., November 1, 1915. Two rows of beans were planted in each bed, each row being over a row of tubes. The rows of beans were 6 inches apart, with 7 hills in each row. Two plants were grown in each hill, making 14 plants in each bed. The beans grew and produced fruit. They are shown in Plate II (fig. 1). The bed on the left is the check bed and contained the jars filled with soil; the bed on the right contains the jars filled with carbon. From the illustration it is seen that the carbon bed has produced the greater growth. The beans were picked and vines cut January 15, 1916, having ceased to produce fruit. The weight of the check bed was 250.0 gm. of vines and 162.5 gm. of beans in the pod and the weight of the carbon jar bed was 390.0 gm. of vines and 250.0 gm. of beans.

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Other experiments of this kind were made by adding harmful organic compounds, namely, vanillin and salicylic aldehyde, to the soil in such amounts as to injure the growth. Porous jars filled with carbon were buried in the soil to determine whether or not the harmful materials would be absorbed from the soil, thereby improving the growth. Vanillin and salicylic aldehyde were selected as the organic compounds for this experiment as they have both been found to exist in some poor soils.

As in the former experiment, the bench beds were 36 inches by 18 inches by 8 inches. In one bed were placed 10 of the porous jars filled with carbon, arranged in two rows of 5 jars each, and in the adjoining bed the porous jars were filled with soil. The soil used in these beds was the silty clay loam, of moderate productivity, from the Arlington Experimental Farm. One of the vanillin treated beds contained porous jars filled with carbon and the other bed jars filled with the silty clay loam. Likewise, one of the salicylic aldehyde beds contained carbon tubes and the other soil tubes. A fifth bed having no treatment and no tubes buried in it was added as a check on the effectiveness of the aldehydes.

Fourteen bean plants grew in each bed. The seeds were planted November 1, 1915. Before planting, 5 gm. of vanillin and 3 gm. of salicylic aldehyde were added and mixed with the soil in their respective beds. Further quantities of vanillin and salicylic aldehyde were added to the surface in a water solution November 12, November 23, and December 3. The material was worked into the soil; each time 5 gm. of vanillin and 3 gm. of salicylic aldehyde were added, making a total of 20 gm. of vanillin and 12 gm. of salicylic aldehyde applied in the respective beds. This makes a total of 200 p.p.m. of vanillin for the two vanillin treated beds, and 120 p.p.m. of salicylic aldehyde for the two salicylic aldehyde treated beds. In Table II is given the yield of vines and pods from the different beds.

Considering the vanillin treated soil first, the growth in the bed which contained the carbon tubes was much better than the checkbed which contained the tubes filled with soil. Comparing the growth in both beds with that in No. 5, it is seen that the vanillin depressed the growth somewhat, but the harmful effect was overcome to a great extent by the absorption of the carbon. The growth in the two vanillin beds is shown in Plate II (fig. 2). The first bed contains the soil tubes and the second the carbon tubes.

The results with salicylic aldehyde were similar to those with vanillin. The growth in bed No. 4, which contained the carbon, was much better than that in the check bed No. 3. By comparing the growth in bed No. 5 with that in No. 3 and No. 4 it is seen that the salicylic aldehyde also produced a harmful effect. This too was partly overcome by the absorption of the carbon in the tubes. In Plate III (fig. 1) are shown the two salicylic aldehyde beds. The second bed contains the carbon tubes.

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A similar experiment was made by growing lettuce in soil treated with salicylic aldehyde. In this experiment one bed contained tubes filled with soil and another bed tubes filled with carbon. The lettuce plants were transplanted November 1, 24 plants of Grand Rapids Curly Leaf variety having been placed in each bed. The amounts and time of application of aldehyde were the same as those given in the experiment with string beans. The lettuce grew rather poorly in this soil. The weight of that from the carbon bed was 645.0 gm. as compared with a weight of 600.0 gm. for the lettuce from the check bed, indicating a slightly better growth in the carbon bed.

TABLE II

EFFECT OF CARBON IN POROUS JARS, ON BEANS IN SOIL TREATED WITH VANILLIN AND SALICYLIC ALDEHYDE

Bed No.	Treatment	Porous jars buried in soil filled with	Green weight gm. Vines	Green weight gm. Pods
1	Vanalin	Soil	172.0	138.8
2	Vanillin	Carbon	225.0	194.0
3	Salicylic aldehyde..	Soil	185.0	139.5
4	Salicylic aldehyde..	Carbon	207.0	194.2
5	Untreated	(No jars)	241.0	209.8

A review of these experiments in greenhouse bench beds shows that in a garden soil in which harmful organic compounds of an aldehyde nature exist and in soil made unproductive by the addition of organic substances, such as vanillin and salicylic aldehyde, carbon incased in porous tubes buried in the soil improves its productivity, presumably by the absorption of the soluble organic substances from the soil solution by the carbon.

Effect of Absorbing Substances Incased in Porous Materials on Growth in the Field

Experiments were also made with carbon in tubes buried in plots in the field. The experiment was enlarged in this case. Several materials, all having absorbing qualities were used, namely carbon black, wood charcoal, chalk ($CaCO_3$) and magnesium carbonate. In addition to using the battery jars, specially constructed concrete tubes and very porous tile drain were used. The cement tubes were made by coating a wire gauze tube with cement, thus making a very thin layer, which is very porous and permits water to pass through freely. These tubes were 2½ inches in diameter and 3 feet long. They were filled with the absorbing material and the ends closed with corks. The other tubes consisted of unglazed earthenware tile drains, which were very porous and permitted water to pass through freely. These were 2¾ inches in diameter and 1 foot long. They were filled and corked at each end in the same manner as the con-

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crete tubes. This porous tubing was buried in the plots before the ground was seeded. Trenches were dug and the tubes laid 8 inches beneath the surface and under the rows where the plants were to grow.

These experiments were made at the Arlington Experimental Farm and in soil which had been growing cowpeas for 6 successive years. These plots are unproductive and produce small yields. The soil is an acid one, having a lime requirement of approximately 2000 pounds of $CaCO_3$ per acre. It requires periodical liming to keep the soil neutral.

Carbon black. Cowpeas were grown in rows 16 feet long and 2 feet apart. Under 4 rows tubes filled with carbon were buried and adjoining them were grown 4 rows as a check, which had no tubes. Some of these tubes were of concrete, some consisted of porous tile drain, and some of battery jars. This experiment was started in the spring of 1914 and 2 years' results were secured. Cowpeas were planted in the spring and grew to maturity, when they were cut and the weight of the cured hay taken. In 1914 the weight of the four rows with carbon tubes was 9 pounds and the weight of the check rows without tubes was 8 pounds, a slight increase of growth in the carbon plot. In 1915 the carbon plot produced 18¼ pounds of cured cowpea hay and the check plot 16 pounds. Both years there was an increase in the carbon plot.

Charcoal. This test was similar to the one with carbon, just described, except that only 2 rows of tubes were used and 2 check rows that had no tubes. The porous tubes were filled with powdered wood charcoal, which was moistened and well packed. The tubes were buried in the soil in the spring of 1914, and cowpeas grown in 1914 and again in 1915. In 1914 the two rows of cowpeas in the charcoal plot produced 6 pounds of cured hay and the check plot produced 3.6 pounds, an increase of 66 per cent for the charcoal plot. In 1915 the charcoal plot produced 12 pounds of dry cowpea hay and the check plot 6.6 pounds, an increase of 80 per cent for the charcoal plot.

Chalk. In another plot chalk ($CaCO_3$) was used in the porous tubes in order to determine whether finely divided chemicals of this character would have a similar effect to those which have only an absorbing effect. Calcium carbonate is practically insoluble in water. In addition to its effect as an absorbent it could have an effect on the soil solution passing through it by neutralizing or precipitating any acids that may be present. Three rows of tubes filled with chalk were buried in the soil, as in the experiment with carbon and charcoal. Cowpeas were grown in the rows in which the tubes were buried and also in three adjoining rows that were to serve as a check. In 1914 the weight of cowpea hay for the chalk plot was 7.0 pounds, and that of the check plot was only 3¾ pounds. In 1915 the differences were not so large; the chalk plot produced 12 2/3 pounds dry weight, and the check plot 10½ pounds. The growth in the first year

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of the experiment is shown in Plate III (fig. 2). The three rows on the left have the chalk tubes; the three rows on the right are without tubes.

Magnesium carbonate. Experiments were made with magnesium carbonate, which is finely divided and, aside from its absorbing qualities, would also produce a chemical reaction with the acids of the soil solution similar to that with chalk. Three rows of tubes were used, cowpeas being grown as before, and the growth was compared with that on three other rows growing beside them.

In 1914 the growth in the magnesium carbonate plot was 14 pounds and in the check plot 14¼ pounds, and in 1915 the weights for the magnesium carbonate plot was 18.0 pounds and for the check plot 17¼ pounds. The magnesium carbonate seems to have had practically no effect, for in the first year there was a reduction of one-quarter of a pound, and in the second year there was an increase of three-quarters of a pound.

The magnesium carbonate and its check plot were, however, on a different part of the farm from the plots on which the other tests were made. The plots with carbon, charcoal, chalk and their checks adjoined each other and the soil conditions were more likely to be similar, whereas the conditions in the part of the field where the magnesium carbonate test was conducted were probably very different. This is indicated by the greater yield in the plot. The effectiveness of the magnesium carbonate in this respect is therefore not ascertained by this test.

The contents of the tubes were examined chemically for absorbed or precipitated material by E. C. Lathrop of this laboratory. The carbon black and charcoal contained a small amount of a liquid fatty acid but no aldehydes could be isolated. The calcium carbonate and magnesium carbonate contained a small amount of fatty acids and showed the presence of aldehydes. The amounts in all cases were too small for further study. The fact that aldehyde reactions were obtained from the carbonates and not from the carbon and charcoal would indicate either a destruction of the aldehydes, possibly by oxidation through absorbed oxygen, or else that the aldehydes, when once absorbed, are tenaciously held by these substances.

SUMMARY

It is pointed out that finely divided carbon is a good agent for physiologically purifying distilled water and certain poor soil extracts, and that by its absorptive qualities it improves the solution as a medium for plant growth.

The test made by mixing carbon black with poor soils failed to effect an improvement, as the carbon, even though it might have had an absorptive action, would itself be intermingled with the soil and be in contact with the plant roots.

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With carbon incased in porous earthenware pots buried in soil, the growth of grass, clover and cowpeas was improved when growing in a poor unproductive soil in the greenhouse.

On benches in the greenhouse a soil which contained salicylic aldehyde and other organic compounds was improved for the growth of string beans by the absorptive action of carbon buried in porous tubes in the soil.

In an experiment with string beans and lettuce in greenhouse benches a soil made poor by the addition of salicylic aldehyde and vanillin was improved in productivity by the action of carbon incased in porous tubes.

In a two years' field experiment carbon, charcoal, and chalk, when put in porous tubes and buried in the plots, caused a good increase in growth of cowpeas.

The beneficial action of carbon and other absorbents may be attributed to its removing something from the soil solution which is harmful to plants. The soil moisture passing through the carbon in its process of moving downward and upward in the soil would be robbed of any such material. Soils which contain soluble organic substances harmful to plants would be improved for crop growth.

Literature Cited

(1) Breazeale, J. F.
1906. Effects of certain solids upon the growth of seedlings in water cultures. *In* Bot. Gaz., v. 41, p. 54.

(2) Livingston, B. E., Britton, J. C., and Reid, F. R.
1905. Studies on the properties of unproductive soils. U. S. Dept. Agr. Bur. Soils Bul. 28.

(3) Livingston, B. E., and others.
1907. Further studies on the properties of unproductive soils. U. S. Dept. Agr. Bur. Soils Bul. 36.

(4) Schreiner, O., and Skinner, J. J.
1914. The harmful action of aldehydes in soils. U. S. Dept. Agr. Bur. Soils Bul. 108.

(5) Shorey, E. C.
1913. Some organic soil constituents. U. S. Dept. Agr. Bur. Soils Bul. 88.

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PLATE I

Fig. 1.—Effect of carbon black in a poor soil on cowpeas. (No. 1 check, No. 2 carbon black buried in the soil in a porous cup).

Fig. 2.—Effect of carbon black in a poor grass soil or clover. (No. 1 check, No. 2 carbon black buried in the soil in a porous cup.)

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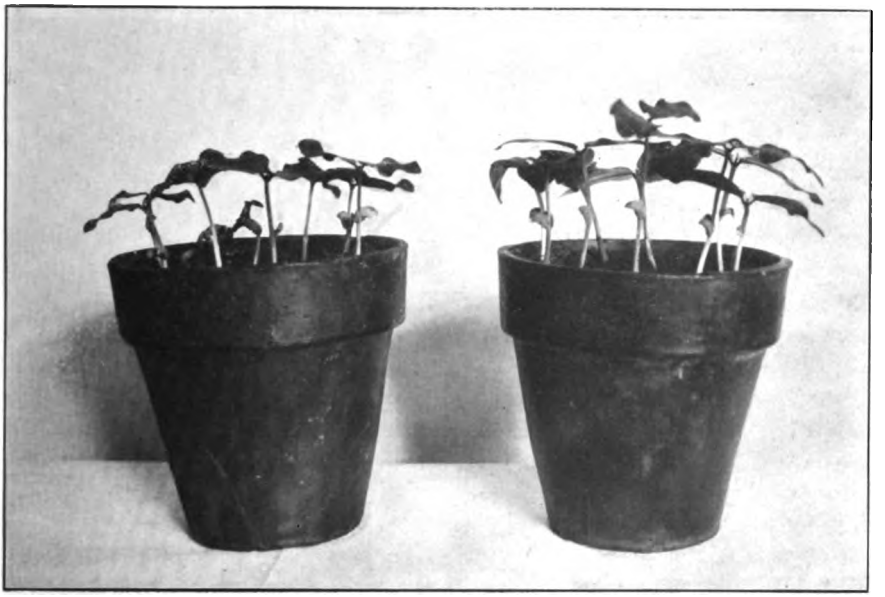

Figure 1

Figure 2

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Figure 1

Figure 2

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PLATE II

Fig. 1.—Effect of carbon in porous jars buried in a poor garden soil, on string beans grown on the greenhouse bench. (Bed on left contains jars filled with soil; bed on right contains jars filled with carbon black).

Fig. 2.—Effect of carbon black in porous jars in a soil to which vanillin was added, on string beans grown on the greenhouse bench. (Bed on right contains jars filled with soil; bed on left contains jars filled with carbon black).

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PLATE III

Fig. 1.—Effect of carbon black in porous jars in a soil to which salicylic aldehyde was added, on string beans grown on a greenhouse bench. (Bed on left contains porous jars filled with soil; bed on right contains porous jars filled with carbon).

Fig. 2.—Effect of chalk in porous jars buried in the soil in the field, on cowpeas. (Jars filled with chalk under the three rows to the left; no chalk under the three rows on the right).

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Figure 1

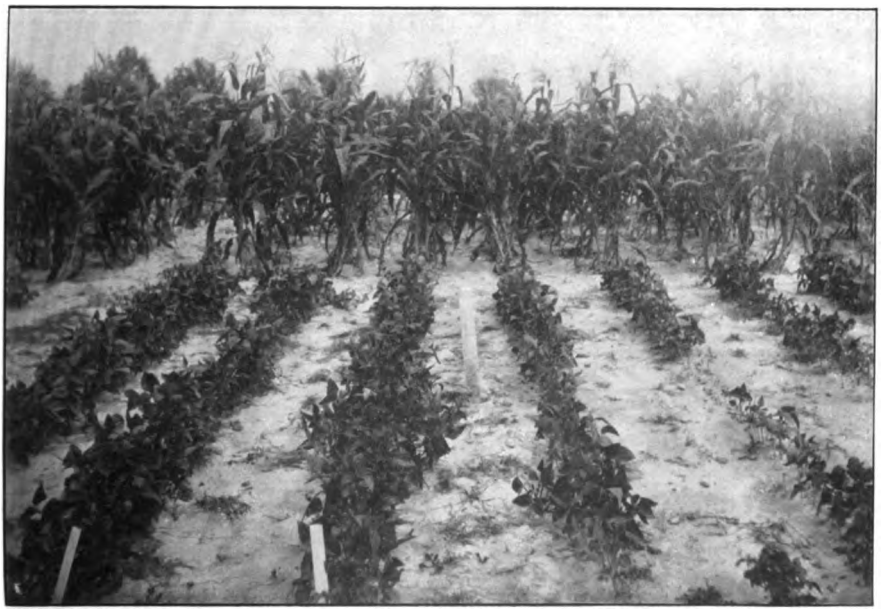

Figure 2

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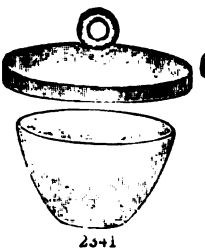

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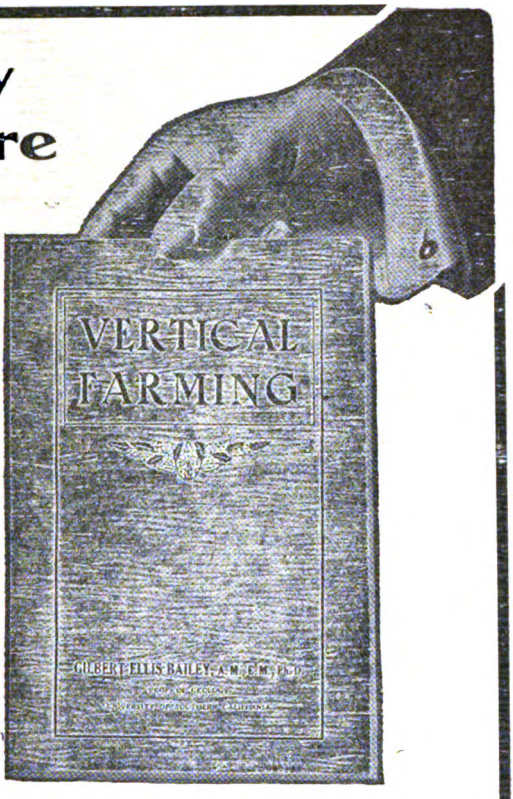

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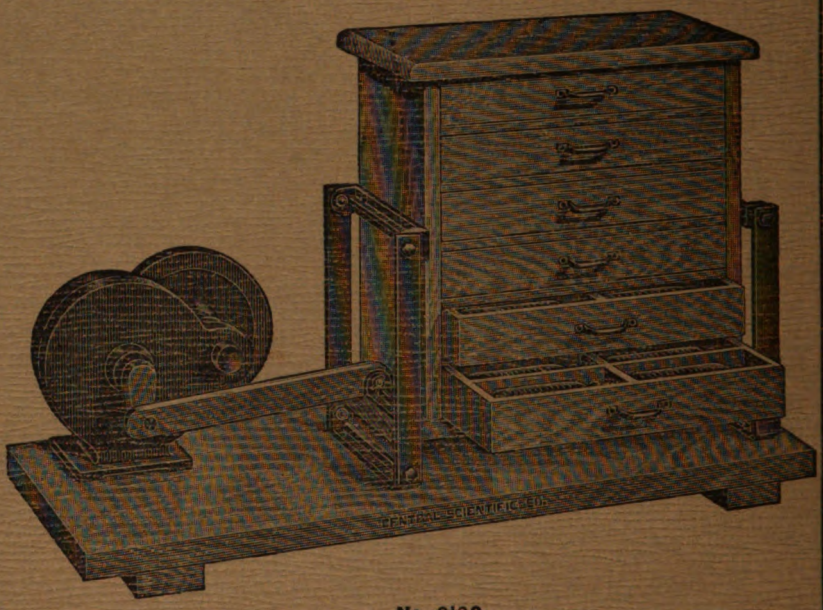

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SOIL SCIENCE

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A · MONTHLY · JOURNAL · DEVOTED TO · PROBLEMS · IN · SOIL · PHYSICS SOIL · CHEMISTRY · AND · SOIL · BIOLOGY

Volume II Number 2

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SOIL SCIENCE

RUTGERS COLLEGE

VOL. II NEW BRUNSWICK, N. J., AUGUST, 1916 No. 2

SOIL FUNGI AND THEIR ACTIVITIES[1]

BY

SELMAN A. WAKSMAN, *Research Assistant, Agricultural Experiment Station, Rutgers College*

INTRODUCTION

It has been recognized for a long time that the soil is a favorable medium for life and life activities. While some investigators have undertaken the study of the flora of the soil proper, others took up the study of the activities of the microörganisms found in the soil, with the idea of penetrating into the secrets of the soil and thus controlling its activities. Since it is now also a well established fact that the largest quantity of plant-food applied to the soil has to undergo certain changes before it can be utilized by higher plants, and that these changes are performed by microörganisms, the knowledge of the activities of these forms of life, the ability to control them, and to direct their activities for the benefit of man, will help to solve the problem of the fertility of the soil.

The author has limited himself in this work to the fungus inhabitants of the soil. But even in this particular group of microörganisms it is impossible and not advisable to study all the forms that could be found in the soil. The question is not, what fungi can be found in the soil, but what fungi are true soil organisms, found not only occasionally and in one soil, but continually and in most soils. What part do these organisms play in the fertility of the soil? The numbers and types of fungi are usually determined, not by those that actually live in the soil, but by the germination of spores on artificial media, having poured plates of high soil dilutions. Many fungus spores germinate slowly, and a soil sample may give numerous colonies of one particular type on a plate, perhaps due to the fact that this organism happened to fruit in the soil sample, or due to the suppression of the slower germinating spores; and the particular organism may not play any great importance in the soil. Hence surveys of the soil flora have to be made repeatedly, at intervals, and this should not be limited to one or two soils, but to a wide area.

[1] Submitted to the Faculty of Rutgers College in partial fulfillment of the requirements for the degree of Master of Science in Research, 1916.

Received for publication May 1, 1916.

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When one speaks of microörganic activities in the soil, the word "bacteria" comes up at once, but the fact should not be forgotten that there are soils, in which the fungi also play a very important part, as is shown by their numbers and activities. The so-called "peat," "acid moor," and other similar soils, which are usually high in humus and have an acid reaction, are very rich in fungi. The mycelium of these organisms is found to penetrate the whole mass of the humus layers of the soil. The grains of a sandy humus soil are often kept tight together by the fungus mycelium. The presence of fungi is not limited merely to the surface, but penetrates deep into the soil. But, as was shown by different investigators, and as will be pointed out later, a rich fungus flora is not limited to acid soils alone, but is found also in neutral, well-aerated, and well-fertilized soils.

HISTORICAL

Adametz (1) was the first investigator who took up the study of soil fungi from a scientific point of view. He isolated his organisms from a loamy and a sandy soil, taking samples at the surface and at a depth of 25 to 30 cm., and came to the conclusion that the two soils harbored the same species, including *Penicillium glaucum, Mucor Mucedo, Mucor racemosus, Mucor stolonifer, Aspergillus glaucus* and *Oidium lactis;* in all he isolated eleven fungi and four yeasts, and did not seem to find any difference in the fungus contents of the different soil layers. Adametz, as well as those who followed him, Reinitzer (85), Nikitinsky (79), and others, studied the fungus flora of the soil for the biochemical activities of the organisms. Nikitinsky (79) isolated from the soil *Penicillium glaucum,* a Mucor and a Trichothecium. Van Iterson (52) isolated a series of fungi from the soil and from the air for the study of their cellulose decomposition, but no differentiation was made between the organisms taken from the soil and those from the air. However, among the forms that he isolated are found several Sporotricha, a Botrytis, a Mycogone, and *Cladosporium herbarum,* all of which are commonly found in the soil.

The first exact study of soil fungi, giving a classification of the organisms and full descriptions, was made by Oudemans and Koning (80) in 1902, in Holland. Forty-five species have been isolated from a humus soil, and among these organisms one finds such forms as *Trichoderma Koningi, Mucor racemosus,* and many others, which have been afterward isolated repeatedly by other investigators.

Following these preliminary investigations, of which the last one only can be considered of real scientific importance in the identification of soil fungi, comes a series of treatises on special groups of organisms. Butler (18) isolated in 1907 several Pythia from the soil, for the study of which he used an Abutillon medium, since these organisms refuse to grow readily on ordinary culture media. Hagem (42, 44) studied several

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soil types, such as arable, meadow, garden, forest and other soils for their content of Mucorales. He found large numbers of these organisms in cultivated soils, though stating that in many cases they give place in numbers to the Penicillia, Aspergilli and Cladosporia. *Mucor racemosus, Mucor hiemalis* and *Mucor nodosus* were found in largest numbers. The *Mucor Ramannianus* and *Mucor silvaticus* group were found predominant in pine forests. Altogether he isolated 18 species, 8 of which were new to science. Hagem (43) stated further that the upper soil layers form a good medium for the growth of the Mucors. Some can live at as low a temperature as 6° to 8° C., and many can develop at 12° to 15° C. The upper temperature limits lie between 27° and 33° C., while the optimum is between 20° and 25° C. Moderate moisture is best for their development. The Mucors do not attack the general organic mass, the carbohydrates and cellulose; they easily attack nitrogen compounds and decompose them. Only a limited number of types assimilate nitrates and nitrites, liberating ammonia. The nitrogen compounds, such as peptones, amino acids, urea and uric acid, are very quickly utilized by the Mucors, as well as by other fungi; ammonia is formed, a part of which is assimilated and built up into fungus proteins; another and larger part does not take place in the growth of the organisms and is set free in the soil. Hagem also found that the cultivated soils vary greatly from the forest soils in their Mucorales content.

Lendner (66) made a study of the soil Mucors in Switzerland the same year as Hagem did his work in Norway. He described several species new to science and combined all the Mucors isolated before into a monograph. Following that came the work of Namyslowski (77, 78), who isolated from the soil *Zygorhynchus Vuilleminii, Mucor microsporus* and *Rhizopus arrhizus*. Ramann (83, 84) stated that he found the strongest growth of fungus mycelium in the fall, when the falling leaves afford rich food and the soil moisture is high; also that the number of fungi in the soil varies within wide limits: as a rule, in a soil that is well aerated, loose and rich in food, the bacteria play the important rôle; in the heavy soils, giving an acid reaction, the fungi predominate. Fischer (35), Hall *et altera* (45), have also pointed out the high content of fungi in the soil.

Beckwith (9), working with "wheat-sick" soils in North Dakota, found the following genera of fungi to be represented there: Fusarium, Colletotrichum, Macrosporium, Alternaria, Spicaria, Verticillium, Rhopolomyces, Cephalothecium and Helminthosporium. As will be seen later, five of these genera were found to be represented in most of the local soils studied by the writer, and had Beckwith found Aspergilli, Penicillia and Mucors, he might have had a picture of many soils, as far as the genera go. Bolley (13), in his study of "wheat-sick" soils found that certain species of Fusarium, Helminthosporium, Alternaria, Macro-

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sporium, Colletotrichum and Cephalothecium are capable of destroying growing plants of wheat, oats, and other crops; this led him to believe that the sickness of the soil is caused by fungi.

Jensen (53) made a fairly complete study of the fungus flora of the soil, taking his samples under sterile conditions and using several soil types and through several seasons of the year. He isolated 35 species, many of which were also reported by previous investigators. Among these are found some of the most common soil groups, such as the Mucors, Penicillia, Aspergilli, Cladosporia, etc. Jensen (53) laid special emphasis upon the occurrence of facultative parasites in the soil.

Dale (27) studied the fungus flora of sandy, chalky, peat and "black earth" soils. The curious thing is that she found in the soil many organisms, such as *Trichoderma Koningi,* several Mucors, Penicillia, Cladosporia and others, which Hagem (42) isolated from the soil in Norway, Koning (80) in Holland, and Jensen (53) in this country. Many of the genera and even the species are the same. She isolated altogether over one hundred organisms, many of which were found in all or in several of the soils.

Goddard (41) isolated 18 species of fungi from the soil for the study of their nitrogen-fixing power. Seven of these organisms are the same as those found by Koning (80). He tried to make a study of the distribution of fungi with soil depth. His results indicate that the organisms were distributed rather uniformly at different depths, at least as low as 14 cm. As to the occurrence of the particular species at the different depths, he did not discover any definite relationship,—any particular species seemed about as abundant at one depth as at another. He was inclined to conclude that there is a rather constant and characteristic fungus flora in the soil, regardless of the treatment as to tillage or manuring.

McLean and Wilson (73) have isolated from a New Jersey Sassafras loam, with an acid reaction, 26 species of fungi, among which are found many organisms reported by the investigators previously mentioned. Finally, mention has to be made of the work of Traaen (108) in Norway, who isolated over 100 fungi from the soil, using filter paper as a substratum. One gets a very faint idea about the organisms that he found, since no identification has been undertaken. He related most of the organisms found to two new genera, subdivided into several species. Of the well known organisms he mentions *Trichoderma lignorum, Trichoderma Koningi* and *Mucor spinosus.*

As seen from the historical review on the occurrence of fungi in soils, the numbers and types of these organisms are large enough to warrant the idea that they play an important part in the fertility of the soil, but to what extent? Are their activities limited to one particular field, or do they take part in the several soil processes which are necessary for the making of a fertile soil? A great deal of work has been done on the

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activities of fungi, as factors in the fertility of the soil, and though some of it was done with organisms isolated from the air or other sources, since these are also found in the soil the work accredited to them may also be understood as taking place in the soil.

The field of fungus activities in the soil can be discussed under the following four headings: first, ammonification; second, nitrogen-fixation; third, nitrogen-transformation; and fourth, cellulose decomposition and humification.

Müntz and Coudon (76) were the first ones to demonstrate the ammonifying power of the several soil organisms, including bacteria and fungi, and it is interesting to note that the two fungi used, namely, *Mucor racemosus* and *Fusarium Müntzii*, gave larger ammonia accumulation in soil than any bacterium used; the same held true in bouillon, with one exception, where one bacterium gave a larger amount of ammonia than the Fusarium.

Marchal (71) proved soon afterward that fungi, such as *Aspergillus terricola, Cephalothecium roseum* and others, are active ammonifiers, especially in acid soils. Butkewitsch (17) pointed out the fact that *Aspergillus niger* is a strong ammonifying organism. Decomposition of urea by fungi has been observed by Miquel (74), Semal (97), and Shibata (99). Perotti (81) found that pure cultures of *Aspergillus fumigatus, Aspergillus ochraceus, Aspergillus niger, Penicillium glaucum, Mucor Mucedo,* and *Botrytis cinerea* showed a good development in dicyanamide solutions, where this was the only source of nitrogen. Kappen (55) found that *Penicillium brevicaule, Stysanus stemonitis,* and two other fungi, were able to decompose cyanamide into ammonia. But dicyanamide could not be utilized by those organisms as a source of nitrogen.

Hagem (43) stated that when ammonia salts are found in the soil, they are quickly utilized and converted into fungus protein,—thereby larger or smaller quantities of easily assimilated nitrogen compounds are taken away from the higher plants and converted into a form which they cannot utilize. In this case the activity of the soil fungi is throughout injurious for the green vegetation. But in the case of dead plants and animal remnants, complicated organic compounds, the soil fungi play a very useful part: they decompose them, liberating ammonia, a part of which may be utilized by them; the larger part, however, is set free, and after being nitrified it can be utilized by the green plants. He ascribed to the soil fungi an important part in the mineralization of organic matter, in which they are greatly ahead of the bacteria.

McLean and Wilson (73) tried out the ammonifying power of a series of fungi isolated from the soil, and concluded that fungi rather than bacteria were responsible for the large accumulation of ammonia under the conditions with which they were working. The Moniliaceae were found to be the strongest ammonifiers, the Aspergillaceae showing the least ammonifying activity. Waksman and Cook (115) thought that

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these differences in the ammonifying power of fungi might be due not so much to the organisms themselves, as to the incubation period used. The Moniliaceae, especially *Monilia sitophila,* producing spores very readily, decompose the organic matter and liberate the ammonia at an earlier period than do the Aspergillaceae, which produce their spores after a longer period. They expressed the idea that there might be some relationship between the periods (e. g. spore production) in the life history of the fungus and the ability to accumulate ammonia in the soil.

The effect of reaction upon ammonification by soil fungi has been recently studied by Kopeloff (60), who found that there exists a comparatively narrow range of reaction tolerance for maximum ammonification, which was found to be between the neutral point and an acidity equivalent to 2000 pounds of CaO per acre.

As to the fixation of atmospheric nitrogen by fungi, a great deal of work has been done on the subject, but the results are still indefinite. Frank (38), Berthelot (12), Puriewitch (82) Saida (91), Remy (87), Ternetz (103), Froelich (40), Latham (64), C. B. Lipman (69), Stahel (101), and Heinze (47), have all shown that fungi are able to utilize free nitrogen. Winogradsky (125), followed by Czapek (26), reported negative results with Aspergilli, and the latter pointed out the possibility of an error in the nitrogen determinations made by Saida (91). Negative results were also reported later by Heinze (49). Duggar and Knudsen (30) found no nitrogen-fixation by fungi, except possibly in certain cultures of *Aspergillus niger.* Kossowicz (61), having tried out the nitrogen-fixing power of a series of fungi, including *Aspergillus niger* and *Penicillium glaucum,* found that they were able to utilize the nitrogen compounds of the air, but were not able to assimilate any free atmospheric nitrogen. Goddard (41) has done about as complete and exhaustive a piece of work on the subject as any previous investigator. He reports negative results for all the forms studied, including at least 14 species, when grown on nitrogen-free media; the amount of free nitrogen taken up from the air by cultures standing exposed, did not seem to be sufficient to make appreciable differences in their nitrogen content, either in nitrogen-free or in nitrogen-containing media. The weight of the conclusions on the nitrogen fixation by fungi seems to lie on the negative side.

The possibility of nitrogen-fixation by another group of soil organisms closely related to the soil fungi, namely, the soil algae, alone, or in association with fungi, has been studied by Schlösing, Jr. and Laurent (96), Kossowitsch (62), Koch and Kossowitsch (57), Stoklasa (102), Beijerinck (10), Heinze (48), and Wilfarth and Wimmer (124).

There is a group of soil fungi for which the ability to utilize atmospheric nitrogen seems to be more or less affirmative, namely, the mycorhizal fungi, but since these organisms do not come within the field of the soil fungi proper, the author will merely refer to the work of Frank

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(36, 37) and Hiltner (50), where references are also given to the other workers on the endotrophic and ectotrophic mycorhiza.

Not a very great deal of work has been done on the utilization of the different forms of nitrogen by fungi, and the transformation of nitrogen compounds. Ritter (89) found that *Aspergillus glaucus, Mucor racemosus,* and *Cladosporium herbarum* can use ammonia nitrogen as well as nitrate nitrogen. Ehrenberg (31) called attention to the fact that soil fungi play a more important part in the building of albuminoids from ammonia nitrogen than the bacteria do. Laurent (65) found *Cladosporium herbarum, Penicillium glaucum, Alternaria tenuis,* and *Mucor racemosus* able to reduce nitrates to nitrites; this did not hold true with *Aspergillus glaucus* and *Aspergillus niger.* But no direct denitrification could be established with fungi, as it is done with bacteria. Ehrlich and Jacobsen (32) found that *Oidium lactis, Monilia sitophila,* Mucors, and other fungi can form oxy-acids from amino-acids.

A great deal of work has been done on the decomposition of carbohydrates by fungi, including celluloses, hemicellulose, pectins, oils, fats, and acids; also on the building up of those complicated compounds in the soil termed humus. Fremy (39) showed in 1848 the ability of *Mucor stolonifer* to destroy pectin. De Bary (8) found that *Peziza libertiana* is able to decompose cellulose. This was also found to hold true for different other fungi by Ward (116, 117), Appel (2), and other investigators which have studied the pathogenicity of certain fungi. Van Iterson (52) distinguishes four ways in which cellulose can be decomposed: by anaerobic bacteria, denitrifying bacteria, aerobic bacteria, and fungi. Among the strongest cellulose decomposers of the 35 distinct species that were able to live on cellulose, he found *Mycogone puccinoides, Botrytis vulgaris,* and a few others. Went (122) found that *Monilia sitophila* is able to secrete cytase. Carbone and Marincola (20) observed the cellulose decomposition by Eumycetes. Carbone (19) found that the Penicillia decompose cellulose; the same thing was pointed out by Christensen (22).

Schellenberg (94) could not find any decomposition by fungi of true cellulose, but found them able to decompose hemicellulose. Dox and Neidig (29) have shown that *Aspergillus niger, Aspergillus fumigatus, Aspergillus clavatus, Penicillium chrysogenum, Penicillium camemberti,* and *Penicillium expansum* are able to destroy the pentosans found in large quantities in the soil and manure. Ohta Kohshi (58) found *Cladosporium herbarum, Penicillium glaucum, Aspergillus glaucus, Aspergillus nidulans* to be able to decompose fats. Roussy (90) proved that fatty acids, namely oleic and palmitic, can be used by fungi as good sources of food.

As to the humifying power of fungi, Frank (37) was the first one to point out the fact that the forest humus is not to be looked upon as a mass of plant remnants, but it is largely a living mass of numerous fun-

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gus hyphae which penetrate in all directions and often make up an important part of the organic substance. They take an active part in the decomposition of organic substances. Höveler (51) looked upon the fungi as the principal factors in humification. Kostyststcheff (63) stated that the microörganisms, including bacteria and fungi, help in the formation of the brown substance of the humus. A similar statement concerning the fungi was made by Scherpe (95). Beijerinck (11) expressed the opinion that the formation of humus in the fields and forests is based upon the equilibrium of oxidations and reductions produced by microorganisms. If oxidation predominates, the humus may disappear; but, if reduction predominates, the soil loses its fertility by the formation of peat.

Koning (59) stated that the Hyphomycetes play a much more important part in the process of humus formation than do the bacteria. He inoculated *Trichoderma Koningi* and *Cephalosporium Koningi* upon sterile leaves covered with a little water. At the end of several weeks the leaves disappeared and the water contained NH_3. Upon the addition of KOH and NH_3 it became dark, as do the humic substances. He further stated that these organisms produce cytase, called also cellulase, which allows them to utilize the cellulose as food. The liquid of the culture gave a faint reaction with Fehling's solution, which led him to conclude that sugar is formed as an intermediate product.

Dascewska (28), having studied both bacteria and fungi from the soil, came to the conclusion that the Hyphomycetes play a more important rôle in the decomposition of cellulose in the soil than do the bacteria. The dark color of humus is probably due, at least in part, to the color of the mycelium and the spores, to brown and black pigments, as well as to oxidizing substances secreted mostly by the Hyphomycetes.

McBeth and Scales (72) concluded that the importance of the filamentous fungi in the soil in the destruction of cellulose has been greatly underestimated. They found that a series of Penicillia, Sporotricha, and other fungi commonly found in the soil were able to decompose different forms of cellulose very rapidly. Scales (93) found that a great number of Penicillia and Aspergilli were able to decompose cellulose, especially where ammonium sulphate has been used as a source of nitrogen.

Heinze (47) stated that soil fungi, the so-called acid builders, play an important part in the making of the insoluble Ca and Mg compounds, soluble and available for plant growth; a similar effect is produced upon the insoluble phosphates, such as Thomas slag, which are made slowly soluble and easily available. A like idea is expressed by Hagem (43), as was already pointed out.

Thus the activities of fungi are found to be manifold; they take an important part in most of the soil processes which work for the keeping up of the fertility of the soil. There are few processes in the soil, such

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as nitrification and nitrogen-fixation, in which the fungi were not found to take an important part, or in which their rôle is doubtful. The problem of the author was to isolate all the fungi from a series of soils and then study their activities, since a great deal of the work reviewed in the previous pages was done with organisms either isolated from the air or living parasitically on plant tissues.

EXPERIMENTAL

Soils Used

Eight soils were used for this work, which will be designated in the following manner:

1. *Garden soil,* a Sassafras sandy loam, on the College Farm, manured every year for the last 20 years with 20 tons of stable manure annually, and receiving an application of lime every 5 years. This soil is almost neutral, showing a lime-requirement, by the Veitch method, of 200 pounds of CaO per acre at 1 inch from the surface, 100 pounds at 4 inches. Below that depth the soil is almost neutral or even slightly alkaline. This soil is used for garden crops by the Botanical Department for experimental purposes.

2. *Orchard soil,* a Sassafras sandy loam, near the garden soil, of exactly the same texture and structure. This soil is the unfertilized plot of the orchard, receiving no application of manure or fertilizer for the last 20 years; only a cover crop of oats has been grown for the last 3 years. Apple trees are grown on this soil, and it receives 8 to 10 cultivations through the summer. This soil has a lower organic matter content than the garden soil, and a high lime-requirement, 2500 pounds CaO per acre at 1 inch from the surface.

3. *Meadow soil,* an Alloway clay, under grass for the last 6 years. This is a heavy soil, with a high moisture, nitrogen, and organic matter content. The lime-requirement is about 500 pounds CaO per acre.

4. *Forest soil,* of the same type as the first two soils, but not cultivated for the last 50 years, if ever before. It contains a large amount of undecomposed organic matter, of an acid character, in the upper 4 inches of soil. Below that depth the soil is very poor in food nutrients, though remaining high in acidity. The lime-requirement of this soil is 4600 pounds of CaO per acre at 1 inch from the surface.

These four soils were the standard soils, from which the samples were taken regularly, under exact methods and at known depths. The description of soil sampling, bacteriological, climatological, and other data will apply to these soils only.

Four more soils have been used for this work, but the data on those soils were merely supplementary, and no exact study has been made of the soils themselves.

5. *Sassafras gravelly loam,* a medium loamy soil, under irregular rotation of crops.

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6. *Iron soil,* a medium loam termed "Colts Neck loam," containing 48 per cent of iron, which gives to the soil a brick-red appearance. This soil is from a peach orchard, near Keyport, N. J.

7. *California soils,* of different texture and structure, taken near Los Angeles.

8. *Oregon soil,* an adobe soil at Corvallis, Oregon.

Samples were taken in all cases under sterile conditions, brought at once into the laboratory, allowing the time of shipment for the last three soils, and plated out on the media used. Care was taken to avoid all possible contaminations from the air, using sterile dishes and sterile water for dilutions. Samples were taken 4 times in the case of the first four soils, namely, September 10, October 21, November 30, 1915, and January 4, 1916, at depths of 1, 4, 8, 12, 20, and 30 inches. The full method of procedure is described elsewhere (113). Samples were taken only once in the case of the last four soils, at a depth of 8 to 10 inches.

Media Used

In choosing the media for the work, the author did not want to make up any new ones, but to select from those used by other investigators such as would give the best results, and that the work might be comparable with that of others. The fault of devising new media every time an experiment is made is great, when one understands the fact that most of the indentification to be done is from descriptions, not comparison of cultures, and since some organisms vary so much with the different media used, one would have to use all the media ever thought of by the different investigators to have results fully comparable. A synthetic medium is wanted, which would have a known chemical composition, and which would be the same wherever used. Such a medium was found in Czapek's agar, used by several investigators, and recommended to the author by Dr. Charles Thom, of the Bureau of Chemistry, U. S. Department of Agriculture. Though many organisms grow very slowly on this medium, the final growth will be characteristic, and a medium which allows a slower development of the organisms is more desirable for the study of the gradual development of the organisms. Other media may be used for supplementary purposes, but one should be used as a standard, with which all the results could be compared.

Four media were used in this work:

1. *Modified Albumen Agar* (16, 113), for the plating out of the original soil samples. It is composed as follows:

Distilled water	1000 c.c.
Dextrose	10.00 gm.
K_2HPO_4	0.50 gm.
$MgSO_4$	0.20 gm.
Egg-albumen	0.15 gm.
$Fe_2(SO_4)_3$	Trace
Agar	15.00 gm.

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This medium was used, not because it is the best one for the isolation of fungi, but for several other reasons: (a) It allowed the greatest development of both fungi and bacteria, under the given conditions; this gave a means of comparison of the numbers of the two most important groups of organisms in the soils used, under absolutely the same conditions. (b) It is a medium rather poor in plant-food, thus allowing only a very slow growth of all the organisms. This made it possible to transfer the organisms when 8 to 10 days old, so that even the slow growing fungi could be isolated without being covered up by the rapidly growing ones. The egg-albumen was dissolved in a little cold water to which a few drops of NaOH were added, and this was added to the hot medium already prepared and well shaken. No coagulation of the albumen will take place upon sterilization. (c) It allows a better development of all different groups of organisms than do the other media, with which this one was compared. (d) The conditions were more uniform when both bacteria and fungi, slowly and rapidly growing forms, could be studied on the one plate. However, no identification was made from this medium, and it was used only for isolation purposes.

2. *Raisin Agar.* This medium was used for the purification of fungi contaminated with bacteria. It was prepared as follows: 60 gm. of yellow raisins were warmed at about 80° C. in 1 liter of tap water for one hour, then filtered, and all the liquid pressed out from the raisins. The filtered portion was made up to 1 liter and 25 gm. of agar were dissolved in it. The medium was then tubed and sterilized. This medium is acid in character, and keeps out the bacteria, thus giving cultures of fungi free from bacteria.

3. *Cook's No. II Medium* (24), composed as follows:

Distilled water	1000 c.c.
Dextrose	20.00 gm.
Peptone	10.00 gm.
K_2HPO_4	0.25 gm.
$MgSO_4$	0.25 gm.
Agar	15.00 gm.

This medium was used for the study of the Mucorales, most of which do not make any good growth upon Czapek's agar.

4. *Czapek's Synthetic Solution Agar,* composed as follows:

Distilled water	1000 c.c.
$MgSO_4$	0.50 gm.
K_2HPO_4	1.00 gm.
KCl	0.50 gm.
$FeSO_4$	0.01 gm.
$NaNO_3$	2.00 gm.
Cane sugar	30.00 gm.
Agar	15.00 gm.

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All the organisms, with the exception of the Mucorales, have been studied upon this medium.

In the process of isolation of the fungi from the soil two methods of procedure have been followed:

1. *The dilution method.* The soil samples, having been taken in sterile flasks, under sterile conditions, at the desired depths, were brought at once into the laboratory. The soil was well mixed and a piece of it was transferred with a sterile spatula into a weighed sterile flask. The flask was then weighed again, and the weight of the transferred soil was found by difference. Dilutions were made up to 2,000, 20,000, and 200,000, sterile water being used. Plates with the albumen agar as the medium were poured from the different dilutions. The plates were then incubated at 22° to 25° C. for 6 to 10 days, after which period the bacteria and fungi were counted, and the fungi were transferred to slants of Czapek's agar with a sterile needle.

2. *The direct inoculation method* (114). Lumps of soil, about 1 c.c. in diameter, were transferred from the sampling flask, with a sterile forceps, into a sterile plate containing 10 c.c. of Czapek's agar. The plates were then allowed to incubate for 24 hours at 22° C. It was believed that in such a short period of time the spores in the soil could not have germinated and formed a mycelium, visible to the naked eye; while the organisms actually living in the soil and forming a mycelium would develop at once and the mycelium would then be visible to the naked eye. After 24 hours of incubation the mycelium was transferred from the plates into sterile slants of Czapek's agar, care being taken to get the mycelium from the medium 1 to 3 mm. away from the soil lump.

The tube cultures from both isolations were allowed to develop for 10 to 15 days, till spore production took place. Single spore cultures were made then, using the raisin agar as the medium for transfer, thus eliminating the bacteria. A small amount of spores and mycelium from the organism in question were introduced into 100 c.c. of sterile water in a 300-c.c. flask. This was well shaken so as to break up the spore material. A platinum needle was then sterilized, dipped into the liquid, and passed several times over the sterile solidified medium in the sterile plate. The plates were then incubated 2 to 3 days till the colonies began to develop. Where the single spores were dropped by the needle, separate colonies could be detected with the naked eye. This was verified by placing the plate, with the lower side upward, under the microscope. The single spore colony, or part of it, was now transferred with a platinum loop into a sterile slant of No. II medium in the case of Mucorales, or Czapek's agar in the case of any other fungus. The tubes were incubated at 22° to 25° C. and growth compared with original growth on Czapek's agar, so as to get the identity of the culture. The last transfer usually gave a culture free from any bacterial or fungus contamination.

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Certain organisms, especially a few Penicillia, always came out from this transfer contaminated with a sterile mycelium, and it took several transfers before such could be had pure.

All the identification and description was done on the last transfer. In the identification of the Penicillia, 15 per cent gelatin in distilled water was also used, as recommended by Thom (104). The media used for cellulose decomposition and diastase secretion by fungi will be described later.

Numbers of Fungi in the Soil

Remy (86), who took his soil samples in different seasons of the year, recorded both the bacteria and fungi developing on the plates. He found no constant ratio between the numbers of the two groups of microörganisms. But on the average he found in cultivated soils ten times as many bacteria as fungi; 1,770,000 to 2,500,000 bacteria and 220,000 to 240,000 fungi were found per gram of soil.

Ramann (83) found an average of 2,400,000 bacteria and 129,000 to 289,000 fungi per cubic centimeter of loamy and sandy soils; in raw humus soil he found 220,000 bacteria and 241,000 fungi per cubic centimeter. Fischer (35) found almost as many fungi as bacteria in cultivated and uncultivated moor lands, but in clay, sandy, and loamy soils the numbers of fungi were much smaller than those of bacteria. In untreated moor land he found an average of 1,600,000 bacteria and 550,000 fungi per gram; when this soil received an application of stable manure, it contained 700,000 bacteria and 1,116,666 fungi per gram. Sandy soil untreated contained 1,450,000 bacteria and 733,333 fungi per gram; manured, 3,650,000 bacteria and 1,816,666 fungi. The writer (112) reported in a previous paper, 6.2 to 7.1 per cent of the total microörganic flora to be fungi, which amounted to 400,000 to 1,100,000 per gram of soil at a depth of 1 inch, 7.9 to 11.7 per cent at a depth of 4 inches, and at lower depths the total number of fungi, as well as their relation to the bacteria and actinomyces, decreased with depth.

As seen from Table I, the cultivated and non-acid soils contain large numbers of fungi, and even larger than those of the forest soil, which is very acid. The numbers of bacteria for those soils throughout the whole year have been reported elsewhere (113). The first three soils were found to contain from 4,000,000 to 20,000,000 bacteria, while the forest soil contained only 500,000 to 4,000,000. The ratio of the bacterial to the fungus numbers in the first three soils was about 10 to 1, while in the forest soil it was about 5-6 to 1, and sometimes the forest soil contained almost as many fungi as bacteria, especially when the medium was slightly acid. When the numbers of fungi found at the different depths are compared, the highest numbers are found at 1 or at 4 inches from the surface. The garden soil contained in three instances out of four larger

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numbers of fungi at a depth of 4 inches than at 1 inch. This was probably due to the high organic matter and moisture content of the lower depth. Below 4 inches the numbers decrease rapidly with depth, the greatest fall usually occurring between 4 and 8 inches. At depths of 20 and 30 inches the numbers of fungi were very small, so that on a 10,000 to 20,000 dilution plate only one or two colonies would develop, and sometimes none at all. This does not agree with Goddard's (41) remark, that the species seemed to be as abundant at one depth as at another.

TABLE I

NUMBERS OF FUNGI PER GRAM OF SOIL AT DIFFERENT DEPTHS[1]

Date of sampling	Depth in inches	Garden soil			Orchard soil			Meadow soil			Forest soil		
		Numbers	Numbers	Average	Numbers	Numbers	Average	Numbers	Numbers	Average	Numbers	Numbers	Average
Sept. 10, '15	1	400	600	500	400	600	500	800	1,000	900	240	200	220
"	4	800	1,000	900	600	1,000	800	1,400	1,200	1,300	160	130	145
"	8	300	200	250	180	120	150	300	200	250	...	...	...
"	12	160	130	145	50	40	45	60	40	50	...	...	...
"	20	60	40	50	30	20	25	30	30	30	...	...	...
"	30	30	20	25	20	10	15	30	20	25	...	...	...
Oct. 21, '15	1	400	400	400	500	400	450	1,200	1,000	1,100	180	240	210
"	4	800	600	700	400	600	500	600	800	700	180	200	190
"	8	600	400	500	100	80	90	120	140	130	...	...	...
"	12	60	40	50	40	20	30	40	40	40	...	...	...
"	20	40	20	30	20	20	20	40	60	50	...	...	...
"	30	40	40	40	0	0	0	20	20	20	...	...	...
Nov. 30, '15	1	200	100	150	400	400	400	600	600	600	240	180	210
"	4	400	200	300	200	100	150	600	800	700	80	100	90
"	8	100	0	50	120	60	90	200	100	150	...	...	...
"	12	40	50	45	60	40	50	60	50	55	...	...	...
"	20	20	30	25	20	20	20	20	10	15	...	...	...
"	30	10	0	5	20	0	10	0	0	0	...	...	...
Jan. 4, '16	1	200	200	200	200	100	150	800	1,000	900	260	200	230
"	4	300	100	200	100	0	50	400	200	300	220	140	180
"	8	120	60	90	40	20	30	200	200	200	...	...	...
"	12	40	40	40	10	0	5	40	40	40	...	...	...
"	20	20	20	20	0	0	0	6	6	6	...	...	...
"	30	2	2	2	0	0	0	2	0	1	...	...	...

[1] Thousands omitted.

The duplicates of the fungus counts checked very poorly, one plate giving 6 to 10 colonies, and the duplicate only 3 to 4 colonies. This might have been due to the fact that the spore material was not broken up well enough when the dilution was made. As to the seasons of the year, no appreciable difference in the fungus content of the soil was found in the different periods of sampling.

In Table II are given all the fungi isolated from the different soils, with special emphasis laid upon the four standard soils. Several more organisms are kept in culture, but the author has not been able to identify

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TABLE II

LIST OF SPECIES OF FUNGI ISOLATED FROM THE DIFFERENT SOILS

Name of Organism	Garden	Orchard	Meadow	Forest	Iron	Oregon	California	Sassafras
Absidia Lichtheimi (Lucet et Const.) Lendner	*	..	..	..	..	..	..	..
Absidia Orchidis (Vuill.) Hagem	..	..	..	..	*	..	..	..
Mucor hiemalis Wehmer	*	*	..	*	..	..	..	*
Mucor microsporus Namyslowski	..	*	..	..	..	..	..	..
Mucor circinelloides van Tieghem	*	*	*	*	..	..	..	..
Mucor plumbeus Bonorden	*	*	*	*	..	*	..	..
Mucor racemosus Fres.	*	*	*	..	..	*	..	..
Mucor silvaticus (?) Hagem	..	*	..	..	..	..	..	..
Mucor botryoides Lendner	..	..	..	..	..	..	..	*
Mucor saturninus (?) Hagem	..	*	..	..	..	..	..	..
Mucor sphaerosporus Hagem	..	*	..	..	..	..	..	..
Mucor sp. (C. 44)	..	..	*	..	..	..	..	..
Mucor sp. (D. 28)	..	..	..	*	..	..	..	..
Mucor glomerula (Bainier) Lendner	..	..	*	..	*	..	..	..
Mucor flavus Bainier	..	..	..	..	*	..	..	..
Zygorhynchus Vuilleminii Namys.	*	*	*	..	*	*	*	..
Rhizopus nigricans Ehren.	*	*	*	..	..	*	..	..
Rhizopus nodosus Namys.	..	*	*	..	..	..	..	..
Rhizopus sp. (B. 3)	..	*	..	..	..	..	..	..
Saccharomyces sp.	*	*	*	*	..	..	..	..
Chaetomium olivaceum Cooke and Ellis	..	..	*	..	..	..	..	..
Chaetonium cochliodes Palliser	..	..	..	..	..	..	..	*
Monilia sitophila (Mont.) Sacc.	*	..	*	..	..	..	..	..
Monilia humicola (?) Oud.	..	..	*	..	..	..	..	..
Oidium lactis Fres.	*	*	*	..	..	..	..	..
Oidium sp.	*	..	..	..	..	..	..	..
Sporotrichum pulvinoforme (?) Thüm	..	..	..	..	*	..	..	..
Sporotrichum roseum (?) Link	*	..	..	..	..	..	..	..
Botrytis cinerea Pers.	..	..	*	..	..	..	..	..
Aspergillus fumigatus Fres.	*	*	*	*	..	*	..	..
Aspergillus nidulans Eidam	*	*	*	*	..	..	..	..
Aspergillus diversicolor Vuill.	*	*	*	*	*	..	*	..
Aspergillus niger van Tieghem	*	*	*	..	..	..	..	..
Aspergillus n. sp. (?) (C. 19)	..	..	*	..	..	..	..	..
Aspergillus glaucus Link	..	..	*	..	..	..	..	..
Aspergillus calyptratus (?) Oud.	..	..	..	..	*	..	..	..
Aspergillus fuscus Schiemann	*	..	..	..	..	..	..	..
Aspergillus flavus Link	..	..	..	..	..	..	*	..
Aspergillus sp. (K. 2)	..	..	..	..	..	..	*	..
Aspergillus repens de Bary	..	..	..	*	..	..	..	..
Aspergillus clavatus Desmasières	..	..	..	..	..	..	..	*
Penicillium luteum Zukal	*	..	..	..	..	..	..	..
Penicillium luteum group Thom	*	..	*	..	*	..	..	..
Penicillium lilacinum Thom	..	..	*	..	..	..	..	..
Penicillium pinophilum (Hedgcock) Thom	..	..	*	..	..	..	..	..
Penicillium purpurogenum, var. *rubrisclerotium* (?) Thom	..	..	*	..	..	..	..	..
Penicillium chrysogenum Thom	..	..	*	..	..	..	*	*
Penicillium commune Thom	..	*	..	..	..	..	..	..
Penicillium decumbens Thom	..	..	..	*	..	..	..	..
Penicillium digitatum Sacc.	*	..	..	*	*	..	..	..
Penicillium expansum (Link) Thom	..	..	..	*	..	..	..	..
Penicillium italicum Wehmer	..	..	..	..	..	..	*	..
Penicillium oxalicum Thom	*	..	..	..	..	..	..	..
Penicillium notatum Westling	*	..	*	*	..	..	..	..

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TABLE II—(Continued)

LIST OF SPECIES OF FUNGI ISOLATED FROM THE DIFFERENE SOILS

Name of Organism	Garden	Orchard	Meadow	Forest	Iron	Oregon	California	Sassafras
Penicillium viridicatum Westling	*	*	..	..	*	..	..	..
Peiicillium atramentasum Thom	*	..	..	..	..	..	..	..
Penicillium glaber[1] Wehmer	*	*	..	*	..	*	..	..
Penicillium Pfefferianus[1] Wehmer	..	..	..	*	..	*	..	..
Penicillium rugulosum Thom	..	..	..	*	..	..	..	..
Penicillium lividum Westling	..	..	..	*	..	..	..	..
Penicillium cyclopium Westling	..	..	..	*	..	..	..	..
Penicillium desiscens Oud.	..	..	*	..	..	..	..	..
Penicillium group I (13-25)	*	*	*	*	*	*	*	..
Penicillium group II (6-22-23)	..	..	*	*	..	*	..	..
Penicillium group III (9-24)	..	..	*	*	..	..	..	..
Penicillium group IV (2-11-15)	..	*	*	..	..	..	..	*
Penicillium group V (18-20)	..	*	..	*	..	..	..	..
Penicillium group VI (10)	*	..	..	..	*	..	..	..
Stopulariopsis brevicaule Sacc. (n. var.?)	*	..	..	..	*	..	..	..
Cephalosporium acremonium Corda	..	..	*	..	..	..	..	..
Cephalosporium curtipes (?) Sacc.	..	..	*	..	..	..	..	..
Cephalosporium sp. (G. 23)	..	..	..	..	*	..	..	..
Cephalosporium sp. (D. 32)	..	..	..	*	..	..	..	..
Cephalosporium sp. (C. 56)	..	..	*	..	..	..	..	..
Trichoderma Koningi Oud.	..	..	*	*	*	*	..	..
Trichoderma lignorum (Tode) Hartz	..	..	*	*	..	..	..	..
Trichoderma sp. (G. 5)	*	..	..	*	*	..	..	..
Trichoderma sp. (C. 10)	..	..	*	*	..	..	..	..
Trichoderma sp. (D. 34)	..	..	..	*	..	..	..	..
Acrostalagmus cinnabarinus, var. *nana* Oud.	*	*	*	..	..	*	..	..
Acrostalagmus albus Preuss.	*	..	*	..	..	..	..	..
Verticillium glaucum (?) Bonorden	..	..	..	*	..	..	..	..
Verticillium terrestre (?) (Link) Lindau	..	..	..	*	*	..	..	..
Cephalothecium roseum Corda	..	..	*	..	..	..	..	..
Zygodesmus sp. (B. 36)	..	*	..	..	..	*	..	..
Dicoccum asperum Corda	*	..	..	..	..	..	..	..
Basisporium gallarum (?) Molliard	..	..	..	..	*	..	..	..
Cladosporium herbarum Pers.	*	*	*	*	..	*	..	..
Cladosporium epiphyllum Pers.	*	..	*	..	..	..	*	..
Dematium pallulans (?) de Bary	..	..	..	..	..	..	*	..
Acremoniella sp. (?) (C. 37)	..	..	*	..	..	..	..	..
Alternaria humicola (?) Oud.	..	..	*	..	..	..	..	..
Alternaria sp. (B. 20)	..	*	..	..	..	..	..	..
Alternaria sp. (A. 36)	*	..	*	..	..	..	..	..
Fusarium angustum Sherb.	..	..	..	..	..	..	..	*
Fusarium bullatum[2] Sherb.	..	..	..	..	..	..	..	..
Fusarium solani[2] Ap. et Wr.	..	..	..	..	..	..	..	..
Fusarium orthoceras Ap et Wr.	..	*	..	..	..	..	..	..
Fusarium oxysporium var. *resupinatum* Sherb.	..	..	..	*	*	..	..	..
Fusarium caudatum Wr.	..	..	..	*	..	..	..	..
Fusarium oxysporium Schlech.	..	..	..	..	*	..	..	..
Melanconium sp. (G. 20)	*	..	..	..	*	*	..	..
Coniothyrium Fuckelii (?) Sacc.	..	..	*	..	..	..	..	..
Sclerotium	..	..	..	*	..	*	..	..
Sterile white mycelium	*	*	*	*	*	..	..	..
Sterile red-brown mycelium	*	..	..	*	..	*	..	..

[1] Organisms belong to Wehmer's genus Citromyces.

[2] Isolated from an alfalfa soil not given in the table.

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them or to determine with any accuracy the species or genus which they approach. Such, for example, are several Mucors, Rhizopuses, Fusaria, Alternaria, Sclerotia, white sterile mycelium producing only chlamydospores, and others.

TAXONOMIC CONSIDERATION OF ALL FORMS FOUND IN THE SOIL

In the identification of the fungi much assistance has been found in Rabenhorst's "Kryptogamen Flora" (34, 68), Engler and Prantl's (33) work on fungi, Thom's (104) and Westling's (123) work on Penicillia, Hagem's (42, 44) and Lendner's (66) work on the Mucorales, and a number of papers on fungi in general and soil fungi in particular, such as the work of Dale (27), Jensen (53), Oudemans and Koning (80), and many others. The measurements are always recorded as found by the author; these in many cases did not coincide with those given in the original description. The identifications being made in most instances after descriptions alone are open to criticism. The arrangement of the descriptions of the fungi has been made according to the classification adopted by Lindau in Rabenhorst's "Kryptogamen Flora" (68).

The author is here taking the privilege of expressing his sincere thanks to Dr. Charles Thom, of the Bureau of Chemistry, U. S. Department of Agriculture, for the identification of several of the Penicillia; to Dr. A. F. Blakeslee, of Cold Spring Harbor, for the determination of the sexual reaction of the Mucors, and determination of several Mucorales; to Dr. A. H. Chivers, of Dartmouth College, for the identification of the Chaetomia; to Dr. C. D. Sherbakoff, of the Florida Experiment Station, for the identification of the Fusaria; to Mrs. F. W. Patterson, of the Bureau of Plant Industry, U. S. Department of Agriculture, for the examination of several organisms; to Dr. M. T. Cook and Mr. C. A. Schwarze, of the New Jersey Agricultural Experiment Station, for different suggestions in the course of the work; and particularly to Dr. J. G. Lipman, Director of the New Jersey Agricultural Experiment Station, for the many helpful suggestions in the outlining of the work.

Phycomycetes

Mucorales

Absidia

Absidia Lichtheimi (Lucet et Constantin) Lendner (A. 30)

This organism forms a white floccose growth upon the No. II medium. The sporangiophores are creeping, terminated by elongated branches, carrying sporangia. Sporangia hyaline, pear-shaped, with an apophysis gradually passing into the stalk of the sporangiophore; it is 22 to 48 μ in diameter, with a smooth membrane, which dissolves easily, leaving a small collarette; columella globose to hemispherical, 14 to 21 μ in diameter, usually smooth or with a short central spine. Spores globose to oval, hyaline, 4 to 6.8 μ in diameter.

Hab. Isolated once from garden soil; also by Lendner in Switzerland.

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Absidia Orchidis (Vuill.) Hagem (G. 24)

Colony dense, brown, 2 to 3 cm. high. Stolons very strongly branched. Sporangiophores are 65 to 250 μ high by 6.2 to 8.8 μ thick, usually in groups of two, often as many as six, always with a septum below the sporangium. Sporangia 17 to 50 μ high and broad, without the apophysis; membrane incrusted with fine granules; the membrane dissolves leaving a rigid collar. Columella conical or hemispherical, spiny or smooth, 10 to 22 μ high and broad. Spores spherical to slightly elliptical, 2.3 to 3.2 μ in diameter, hyaline singly, but weak-yellow in mass (Pl. I, fig. 1-3).

Hab. Isolated from iron soil; also by Hagem from Norway soils.

Mucor

This group of organisms forms together with the Penicillia and Aspergilli the three dominant groups of soil fungi in total numbers and in variety of species. One is most likely to find this group in greater abundance in the soil, than any other group of soil organisms, because the Mucors grow readily on most of the media used for the isolation of soil forms, and will easily overrun the other organisms. But if media are used, such as Czapek's agar, which will not favor the rapid development of the Mucorales, the Penicillia will be found to be even more widely spread in the soil than the Mucors. As to types and numbers in the soil, this group is very important. Hagem (42, 43, 44) did a great deal of work on the Mucors of the soil in Norway. He divided them into classes as to their common associative occurrence. It is characteristic that the *Mucor Ramannianus* group, found by Hagem to be one of the most common groups of soil Mucors, has been isolated by the writer only to a very small extent. *Mucor Ramannianus* has not been isolated at all and *Mucor silvaticus* was isolated only once from the orchard soil.

The common groups of Mucors in the soils studied were found to be the *Mucor circenelloides, Mucor plumbeus, Mucor racemosus,* and *Zygorhynchus Vuilleminii.* These four groups have been found in most of the soils studied, at every isolation; and it seems peculiar that these four organisms, or groups of organisms, seem to be among the very few which lead an active life in the soil, as was shown by the direct inoculation method.

All the Mucorales have been studied on the No. II medium.

Mucor hiemalis Wehmer

Colony at first gray, later becoming brownish, 1 to 3 cm. high. Sporangiophores at first erect, later bending down, unbranched in young cultures, with some branching after culture is 10 to 12 days old. Sporangia yellow-brown with a tinge of green, globose, 52 to 106 μ in diameter; sporangial wall dissolving, leaving a small collarette. Columella globose to somewhat oval, 32 to 52 μ high and 23 to 40 μ broad. Spores elliptical, 4.5 to 8 x 3 to 5.2 μ. Chalmydospores numerous, globose to barrel shaped, 10 to 14 μ in diameter, occurring sometimes in the sporangiophores (Pl. II, fig. 7-9).

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Hab. Isolated several times from the garden, orchard and forest soils; by Hagem in Norway, Lendner in Switzerland, Namyslowski in Austria, Jensen in Ithaca, and McLean and Wilson in New Jersey.

Mucor microsporus Namys. (B. 12)

The general characters of this organism, when grown of the No. II medium, coincide very closely with those given by Namyslowski (77).

Hab. Isolated once from the orchard soil; by Namyslowski in Austria, and McLean and Wilson in New Jersey.

Mucor circinelloides van Tieghem (C. 29)

Colony at first gray, later becoming gray-brown to brown, 1 to 1.5 cm. high. Sporangiophores, up to 1 cm. high, branched sympodially, usually with regular branching to right and left; side branches are slightly bent, all ending in sporangia. Sporangia spherical, gray-brown when mature, 35 to 67 μ in diameter; wall dissolving easily in the case of the lower, larger sporangia, but the upper, smaller ones, often remain intact; wall is incrusted with fine grains, and leaves a basal collar, when dissolving. Columella globose to half-globose, colorless, smooth, 14 to 35 μ in diameter. Spores globose to elliptical, 3.5 to 5.2 x 3.2 to 3.8 μ. Chlamydospores intercalary, hyaline, smooth, spherical to oval, 10 to 19 μ in diameter. This organism was found by Dr. Blakeslee to give a (—) or male reaction (Pl. II, fig. 4-6).

Hab. Found to be one of the most common species in the soils examined. It has been isolated about 100 times from all the local soils, also from the Oregon and California soils. The wide distribution of this organism is also associated with its life-activity in the soil; also by Hagem in Norway, Dale in England, and Jensen in Ithaca.

Mucor plumbeus Bonorden
Syn.: *Mucor spinosus* van Tieghem

This organism, as well as the *Mucor circinelloides,* forms a very common and widely distributed soil organism. But it varies greatly in size and shape of columella and spores, so, the author believes, that this is not one single species, but it embraces a group of organisms, having a spiny columella. This fact seemed to have been recognized already by Lendner (66), who formed a new species—*Mucor spinesceus.* A similar idea concerning this organism has been expressed by Dr. Blakeslee. Colony black-brown, up to 2 cm. high. Sporangiophores 7 to 10 μ in diameter, upright, rigid, mixed monopodially and cymose-symbodially branched, each branch ending in a sporangium and having a septum at the base. Sporangia brown, spherical, with dissolving wall. No sexual reaction was found in any of the strains (Pl. II, fig. 10-13).

Strain I (D. 23). Sporangia 40 to 60 μ in diameter. Columella short cylindrical, brown, with a basal collar, having 1 to 3 spines 16 to 35 x 10 to 38 μ, or none at all. Spores globose to somewhat elliptical, 5.2 to 9.2 μ in diameter, yellowish when single, brown in mass.

Strain II (C. 22). Sporangia and columella are quite variable in size. Sporangia dark brown, 42 to 86 μ in diameter. Columella oval, subcylindrical, and cylindrical, 20 to 32 x 14 to 19 μ, bearing a variable number of spines. Spores spherical, 7 to 8 μ in diameter. Chlamydospores cylindrical to egg-shaped, 7 x 14 μ.

Strain III (C. 20). This organism closely approaches Lendner's form *Mucor spinesceus.* Low gray growth, 1 to 2 mm. high, becoming dark to almost black with age. Sporangiophores rigid, sometimes slightly curved. Sporangia globose,

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18 to 60 μ in diameter, having a somewhat spiny membrane which dissolves, leaving a basal collar. Columella globose to cylindrical, oval or pyriform, spiny, 7 to 18 x 10 to 35 μ. Spores globose to oval, 4 to 6 x 5 to 7 μ, yellowish in color. Chlamydospores spherical to barrel-shaped.

Hab. This organism, or rather group of organisms, has been isolated from the garden, orchard, meadow, and California soils; also by Hagem in Norway, Jensen in Ithaca, and Dale in England.

Mucor racemosus Fres. (A. 31)

Colony gray at first, later becoming brown, 1 to 2 cm. high. Sporangiophores varying greatly in length, 15 to 24 μ thick, unbranched when young, then branched monopodially with numerous chlamydospores, singly or in chains, spherical, angular or cylindrical, set free when ripe. Sporangia greatly varying in size, globose, yellow-brown, with a smooth, non-dissolving membrane, 70 to 140 μ in diameter. Columella oval to egg-shaped, somewhat cylindrical at the base, always higher than broad, 38 to 65 x 52 to 140 μ. Spores oval, 3 to 6 x 6 to 9 μ.

This organism gave no sexual reaction.

This species should be looked upon also as a group of organisms rather than as a single species, for the abundance and great variability of the different strains, especially when isolated from different soils.

Hab. Isolated from garden, orchard, meadow, and Oregon soils; also by Adametz in Germany, Lendner in Switzerland, Hagem in Norway, Dale in England, Jensen in Long Island, and McLean and Wilson in New Jersey.

Mucor silvaticus (?) Hagem (B. 15)

Colony at first white, then gray. Sporangiophores 1 to 2 cm. high, branching sympodially, the stalk being separated from the further growing branches by a septum. Sporangia never sessile, light brown, globose, 40 to 60 μ in diameter, with smooth, easily dissolving wall. Columella globose, hyaline, 12 to 38 μ in diameter, with a basal collar. Spores elliptical to cylindrical, 2.3 to 2.8 x 3.5 to 5.2 μ. Chlamydospores lemon-shaped, yellowish, 14 x 18 μ. Oil drops found abundantly in hyphae.

This organism gave a (+) or female reaction.

Hab. Isolated once from orchard soil; also by Hagem in Norway.

Mucor botryoides Lendner

Colony at first light brown, later becoming grayish-brown, consisting of a cobweb-like network, up to 2 cm. high. Sporangiophores bending, 15 to 18 μ in diameter, ending in a large sporangium, with a cluster of branches below, each terminated by a sporangium. Sporangia globose, at first gray, then turning yellow-brown, with dissolving membrane; terminal sporangium up to 75 μ in diameter. Columella oblong, 12 to 35 x 17 to 52 μ, hyaline. Spores globose, smooth, 5.2 to 9.8 μ in diameter (Pl. I, fig. 4, 5).

Hab. Isolated several times from Sassafras loam; also by Lendner in Switzerland, Jensen in Ithaca, and McLean and Wilson in New Jersey.

Mucor saturninus (?) Hagem (B. 6)

Colonies at first lead-gray, later becoming black. Sporangiophores varying in height from 1 mm. to 2 cm., branched monopodially and sympodially. Sporangia finally becoming black, 25 to 150 μ in diameter. Columella oval, globose, to egg-shaped, 10 to 52 x 15 to 68 μ. Spores spherical to somewhat elliptical, 4 to 5 x 4 to 8 μ in diameter (Pl. I, fig. 6-8).

Hab. Isolated from the orchard soil; also by Hagem in Norway.

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Mucor sphaerosporus Hagem (B. 22)

Colony yellow-brown with a grayish tinge. Sporangiophores differing greatly in height, 0.2 to 1.2 cm., the longer ones usually sympodially branched. Sporangia globose, yellow-brown, 35 to 85 μ in diameter; sporangial wall encrusted with fine grains and breaking up into small fragments, which cling to the spore masses. Columella oval, slightly attenuated at the base, 25 to 58 x 27 to 67 μ. Spores spherical, smooth, 5.2 to 9.8 μ in diameter. Chlamydospores abundant, occurring both on the vegetative hyphae and sporangiophores. Reaction of culture is (—) or male.

The culture of this organism agrees closely with the one received from Miss Dale, but the abundant large spores were not found in it.

Hab. Isolated from the orchard soil; also by Lendner in Switzerland, Hagem in Norway, and Dale in England.

Mucor sp. (C. 44)

Colonies low and slow growing, gray-black to black. Sporangiophores 200 to 3000 μ high by 8 to 14 μ thick, septate, branched sympodially and monopodially, some branches being very short and carrying circinate sporangia. Sporangia globose, black, 30 to 90 μ in diameter, wall fracturing and leaving a basal collar. Columella hyaline, variable in size, usually egg-shaped to spherical, 10 to 45 x 15 to 52 μ. Spores spherical, gray, 3.5 to 4.4 μ in diameter (Pl. I, fig. 10, 11).

Hab. Isolated once from the meadow soil.

Mucor sp. (D. 28)

Colony forms at first a solid gray growth, which becomes later yellowish-brown to dark gray; hyphae creeping, filled with oil globules, 6 to 8 μ thick. The sporangiophores form loose tufts, up to 1.5 cm. high, 6 to 35 μ thick, monopodially branched, with numerous chlamydospores, and rough surface. Sporangia 40 to 170 μ in diameter, yellow in color, later becoming black; wall breaking, leaving a small collar at the base of columella. Columella oval, higher than broad, and broader at the base than at the top, 20 to 45 x 25 to 88 μ. Spores usually egg-shaped, large, 5 to 8 x 6 to 12 μ, of a yellowish color. Chlamydospores very abundant, yellow, spherical, egg-shaped, or cylindrical, 17 to 26 μ in diameter (Pl. I, fig. 12-14).

Hab. Isolated once from the forest soil.

Mucor glomerula (Bainier) Lendner (C. 4)

This organism coincides very closely to (C. 8) received from Miss Dale. Colony brownish-white, up to 3 cm. high. Sporangiophores, 13 μ in diameter, form a cluster of short branches, or end in a terminal sporangium, surrounded by a verticil of secondary sporangia. Sporangia spherical, dark brown, with fine hairs on the surface, 25 to 72 μ in diameter; wall dissolving, leaving a basal collar. Columella smooth, flattened or subcylindrical, 16.4 to 18 x 21 to 28 μ or 44 x 36 μ when subcylindrical. Spores smooth, globose, 3.2 to 5.6 μ in diameter. Chlamydospores spherical to elliptical, 4.6 to 5.4 x 4.8 to 8.8 μ. The formation of branching, rhizoid-like sporangiophores in contact with the glass of the tube is very characteristic (Pl. II, fig. 1-3).

Hab. Isolated from meadow and iron soils; also by Lendner in Switzerland, and Dale in England.

Mucor flavus Banier (G. 2)

Colony at first white, later becoming gray, up to 2 cm. high. Mycelium is filled with large oil globules. Sporangiophores branched monopodially, yellowish color,

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12 to 30 μ thick; sporangia yellow, 48 to 205 μ in diameter, with easily dissolving wall. Columella ovate, 37 to 52 μ broad, 41 to 95 μ high; spores elliptical to spindle-shaped, hyaline, 2.7 to 4.4 x 5.3 to 9.6 μ.

Hab. Isolated from iron soil; also by Hagem in Norway, and Lendner in Switzerland.

Zygorhynchus

Zygorhynchus Vuilleminii Namys.

This organism is found to be one of the most common soil fungi, having been isolated from all the soils studied. It is very easily isolated, when a sample of subsoil, 12 to 20 inches deep, is inoculated directly into a plate of sterile medium. This organism will be one of the very few developing out of the soil upon direct inoculation.

CZAPEK'S AGAR. A creeping, much branching mycelium, without any surface growth, except the sporangial masses.

MEDIUM No. II. White floccose growth, becoming gray, then black from the mass of zygospores. Sporangiophores hyaline, smooth, pendant or upright, septate, 100 μ long and 8 to 10 μ thick. Sporangia spherical, 30 to 90 μ in diameter, yellow to waxy-brown, with a fine, spiny membrane. Wall dissolving and leaving a basal collar. Columella spherical to somewhat flattened, 22 to 28 (12 to 43) μ in diameter. Spores elliptical to cylindrical, 1 to 2.5 x 4 to 4.4 μ, size varying greatly with strain isolated. Chlamydospores single or in chains, globose, egg-shaped, or cylindrical, 12 to 18 x 15 to 22 μ. Zygospores formed in large numbers, 21 to 43 μ in diameter, of a deep brown color and covered with short tubercules (Pl. III, fig. 1-5).

Dr. Blakeslee identified this organism as *Zygorhynchus Moelleri* Vuill., closely related to *Zygorhynchus Vuilleminii* Namyslowski.

Hab. Isolated from all the soils examined by the writer; also by Namyslowski in Austria, Hagem in Norway, McLean and Wilson in New Jersey, and Jensen in Long Island.

Rhizopus

Rhizopus nigricans Ehrenberg

This organism agrees closely with the one received from Dr. Blakeslee.

Hab. Isolated from garden, orchard, meadow, and Oregon soils also by Adametz in Germany, Hagem in Norway, Jensen in Ithaca, and McLean and Wilson in New Jersey.

Rhizopus nodosus Namys. (B. 5)

Identified by Dr. Blakeslee.

Hab. Isolated from orchard and meadow soils; also by Lendner in Switzerland, and Hagem in Norway.

Rhizopus sp. (B. 3)

Several more species of Rhizopus have been isolated from the orchard and other soils. No attempt was made to identify them, for the lack of information on this genus, as well as the lack of time to carry on extensive biochemical investigations, which would help to distinguish them. The work of Hanzawa (46) is a step forward in the study of this genus. The form (B. 3) was found by Dr. Blakeslee to be neutral, with no sexual reaction.

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Ascomycetes

Saccharomyces sp.

Several species of yeasts have been isolated from the different soils, including yellow, orange, and white species. But no effort was made to identify them.

Chaetomium olivaceum Cooke and Ellis

The description of this organism closely agrees with the one given by Jensen (53).

Hab. Isolated once from meadow soil; also by Jensen in Ithaca.

Chaetomium cochliodes Palliser

This organism has been identified by Dr. Chivers.

Hab. Isolated once from Sassafras loam.

Fungi Imperfecti

Hyphomycetes

Mucedenaceae

Monilia

Monilia sitophila (Mont.) Saccardo (C. 25)

Colony white, floccose, spreading. Red masses of conidia appearing on the margin, usually at the rim of the Petri dish; the growth on a slant is far above the substratum, close to the glass of the tube. Vegetative hyphae hyaline, greatly branched, septate; surface mycelium carrying short branches, from which the numerous conidia are formed. Conidia egg-shaped to cylindrical, 5.2 to 13.4 μ in diameter; they are formed by the rounding off of the cells separated by partitions from the mother cell; conidia give rise to new ones by budding.

Hab. Isolated from garden and meadow soils; also by McLean and Wilson in New Jersey.

Monilia humicola (?) Oudemans (C. 42)

Colony consists of loosely interwoven hyphae, colorless when young, green when old, creeping, branched, septate, 4.8 to 5.2 μ in diameter. Fruiting hyphae are produced as alternate or opposite side branches of the vegetative hyphae and are closely septate. Conidia yellow to greenish, smooth, elliptical, 2.4 to 3.6 x 2.7 to 5.8 μ (Pl. III, fig. 6).

Hab. Isolated once from the meadow soil; also by Koning in Holland.

Oidium

Oidium lactis Fres. (A. 22)

Growth forms a white, slimy cover over the medium. Mycelium consists of irregularly branching, septate hyphae; conidia are formed by the separation of the oidial bodies by partition walls. The whole hypha may break down into single cells, which are not rounded at the ends. Chains of oidia often form a zigzag line, because the individual spores still stick to one another; spores 4.4 to 6. 4 x 6.2 to 8.8 μ. Chlamydospores are also found (Pl. IV, fig. 14).

Hab. Isolated several times from garden, orchard, and meadow soils.

Oidium sp. (A. 30)

Colony white floccose, up to 2 cm. high; at the base of the growth, where it starts from the substratum, it is of a dirty green color, later becoming brown;

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reverse of colony is dark green, substratum becoming colored brown. Mycelium consists of irregularly branching hyphae, which break up easily into cylindrical cells, with edges only slightly rounded at the corners. Cells differ greatly in size, 5.2 to 8.5 x 6 to 35 μ. Contents hyaline or slightly granular. No actual budding was found to take place, only some spores have shown slight swelling on the sides, which reminded one of budding (Pl. IV, fig. 13).

Hab. Isolated once from the garden soil.

Sporotrichum

Sporotrichum sp. (pulviniforme ?) Thüm (G. 6)

Colony white, spreading, cushion-like, with a slightly slimy surface. Hyphae creeping, hyaline, septate, little branched, 2 to 2.5 μ in diameter. Conidiophores arising as short side branches up to 20 μ long. Conidia formed in groups on the sides of the hyphae, or on the tips of the branches; elongated, rounded at the ends, 2.2 to 3 x 4 to 6 μ, with two oil drops in each.

Hab. Isolated twice from the iron soil.

Sporotrichum roseum (?) Link

This form coincides very closely with that described by Miss Dale under (D. 5).

Hab. Isolated once from the garden soil; also by Dale in England.

Botrytis

Botrytis cinerea Pers. (C. 28)

Colony gray to gray-green, finally becoming dark to almost black, surface gray from the conidial masses. Mycelium hyaline, branched, septate, 3.5 μ in diameter. Conidiophores erect, septate, branching only at the tip, 11 to 18 μ in diameter. Conidia form a thick head around projections of the top branches; they are soon separated, ovate to elliptical, finely apiculated at the base, 10.2 to 17 x 7 to 8.5 μ, with hyaline membrane.

Hab. Isolated once from meadow soil; also by Lendner in Switzerland.

Aspergillus

This genus is in some soils most representative in numbers, though not found in such a large number of species as the genus Penicillium. Some 18 species have been isolated from the soil, but only 12 of them are described here; the others are still under observation.

Aspergillus fumigatus Fres. (C. 18)

This organism is similar to the one described by Lindau (68).

Hab. Isolated repeatedly from all the local soils, from the Oregon soil; also by Jensen in Ithaca.

Aspergillus nidulans Eidam

Closely coincides with Lindau's (68) description.

Hab. Isolated repeatedly from all the local soils; also by Goddard in Michigan.

Aspergillus diversicolor Vuill.

Colony at first green, later changing to a rosy tinge, and finally the whole colony becomes rose colored, with the production of a soluble red pigment. The green color always appearing first, the rose color developing only later, never to become green again. In some strains, after many transfers, the rose color appeared

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at once, without any green coming in its successive stages. Vegetative hyphae, 1.6 to 2.1 μ in diameter, filled with oil globules; fertile hyphae 300 to 500 x 4.4 to 6.2 μ. Swelling spherical to somewhat elongated, 13 to 16 x 17 to 23 μ, closely beset with branching sterigmata; primary sterigmata 4.4 to 9.2 x 3.5 μ; secondary sterigmata 4.6 to 8.8 x 2.4 to 2.8 μ. Spores globose, warty, 2.7 to 3.4 μ in diameter.

Hab. Isolated repeatedly from all the local soils, and also from the iron and California soils.

Aspergillus niger van Tieghem

Mycelium at first white, then becoming canary-yellow. All the forms isolated produce the yellow color in the substratum. Conidiophores up to 2 mm. long and 8.5 to 15 μ in diameter; swelling globose, 35 to 43 μ in diameter. Primary sterigmata 16 to 19 x 4 to 4.5 μ, carrying 3 to 4 secondary sterigmata 8 x 3 μ, each ending in a long chain of spores. Conidia smooth at first, becoming warty with age, brownish when single, deep brown to black in mass; spherical, thick-walled, uniform in size, 3.5 to 3.8 μ in diameter. Sclerotia at first yellow, then becoming brown, 166 to 300 μ in diameter.

The different strains of this organism are quite distinct from one another, the above description being based upon the strain (B. 16), which approaches the general description of *Aspergillus niger* more closely than any other strain; (C. 28) for example, formed spores 4.2 to 4.6 μ in diameter.

Hab. Isolated from garden, orchard, and timothy soils. A common organism in the soil, though not so widely distributed as the first three Aspergilli.

Aspergillus (n. sp. ?) (C. 19)

Colonies of a golden yellow color, with a brown reverse, and no pigment production in the substratum. Sterile hyphae creeping, branched, septate, yellowish in color; margin of colony is large, transparent, and spreading. Conidiophores unbranched, unseptate, arising directly from the substratum or as side branches of the vegetative aerial hyphae, have a tendency to bend slightly right below the fruiting body, 200 to 450 (50 to 1000) μ high by 8.8 to 13 μ thick, terminating in a globose swelling; the surface of the conidiophore is rough and brown in color. Swelling brown in color, 31 to 44 (17.5 to 52) μ in diameter, closely beset with simple radiating sterigmata, 5 to 17 x 3.2 to 3.6 μ; together with the simple sterigmata, branching ones occur; the primary sterigmata are then 13.2 x 6.2 μ, secondary 7.2 x 3.5 μ. Conidia yellowish when single, red-brown in mass, globose, 3.5 to 4.8 μ in diameter, and forming long chains. Germination of the conidia takes place through one tube. Gelatin is liquefied in 15 days at 18° C. Chlamydospores present in the vegetative hyphae, 4.4 to 10.2 μ in diameter (Pl. III, fig. 7, 8).

Hab. Isolated several times from timothy soil.

Aspergillus calyptratus (?) Oud. (G. 15)

Colony red-brown, with a yellowish spreading margin; reverse brown, slightly coloring the medium. Vegetative hyphae hyaline, septate, 1.8 to 2.3 μ thick; conidiophores arising directly from the substratum or as side branches of the vegetative hyphae, smooth, hyaline, septate, 42 to 170 μ high and 4.4 to 5 μ thick. Sterigmata simple, pointing forward, very close, numerous, cylindrical, 2.1 to 2.6 x 5 to 7.2 μ. Conidia globose, 2.4 to 2.8 μ in diameter, smooth, vacuolated, hyaline singly, brown in mass, born in long chains and forming a dark cylindrical head, 42 to 56 x 80 to 170 (40 to 83 x 80 to 425) μ. Swelling ellipsoidal to obpyriform, 7.1 to 13.2 x 12 to 18 μ. Chains of spores not broken up readily when mounted (Pl. IV, fig. 1, 2).

Hab. Isolated from iron soil; also by Koning in Holland.

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Aspergillus fuscus Schiemann (A. 29)

This organism agrees very closely with the culture received from Dr. Thom.

Hab. Isolated from garden soil.

Aspergillus flavus Link (N. 41)

Colonies bright green, at first light, then darker, edge of colony yellowish brown. Vegetative hyphae hyaline, 2.2 to 2.7 μ in diameter; conidiophores 500 to 1500 μ high by 8.5 to 17 μ thick, surface rough, colorless. Swelling spherical, 26 to 31 μ in diameter, with a rough surface and yellow-green contents. Conidial head up to 400 μ in diameter. Sterigmata unbranched, radiating, 3 to 3.6 x 12 to 15 μ. Conidia bright green, globose, 3.2 to 5.2 μ in diameter. Long chains of spores not broken easily when mounted. Perithecia not observed. This culture compares favorably with the one received from Dr. Thom, as the Amsterdam strain.

Hab. Isolated from California soil.

Aspergillus sp. (N. 40)

Colony snow-white, spreading, reverse creamy; there is a scant production of conidial fructifications and an abundant formation of sclerotia, produced in large numbers when culture is about 8 to 10 days old. Conidiophores 250 to 300 (165 to 1100) μ long by 5.4 to 10.6 μ thick, arising directly from the substratum or as side branches of aerial hyphae; surface rough, yellowish. Sterigmata pointing forward, forming a solid head, 42 to 175 μ long by 80 to 100 μ thick, thinner at the base than at the top. Primary sterigmata 1.8 to 2.6 x 7.1 to 10.6 μ, secondary 2.2 to 2.7 x 5.4 to 7.8 μ. Conidia yellowish, spherical, 2.2 to 3.4 μ in diameter. Swelling globose to oblong, 10 to 30 x 18 to 35 μ. Sclerotia spherical and subcylindrical, yellow in color, 125 to 800 x 165 to 1000 μ.

Hab. Isolated from California soil.

Aspergillus clavatus Desmasières

Colony gray-green, surface powdery; reverse of colony brownish. Vegetative hyphae hyaline, 2.1 to 2.8 μ thick; conidiophores 400 to 3000 μ high by 21 to 26 μ thick, marginal ones shorter and white, while central ones are tall and lead-gray. Fruiting bodies at first elongated, 65 to 132 x 125 to 300 μ, later becoming more rounded; swelling club-shaped, 35 to 62 x 200 to 350 μ, surrounded by simple, radiating sterigmata, 2.6 x 4.6 to 8.8 μ; conidia oval-shaped, 2.1 to 2.8 x 2.7 to 3.6 μ. By selection the author has been able to isolate two strains from the original, differing only in the length of conidiophores, one having average length of conidiophores over two times as long as the other.

Culture agrees in all respects with the one received from Dr. Thom.

Hab. Isolated once from Sassafras loam.

Aspergillus repens de Bary

The general characters of this organism are exactly the same as for *Aspergillus glaucus,* but all the parts are smaller in size; this point places it with the *"repens."* Colony at first light green, changing to dark green, and soon becomes covered with golden-yellow perithecia. Conidiophores 85 to 250 μ long by 8.8 μ thick. Swelling spherical, 22 to 31 μ, carrying unbranched sterigmata, 3.6 x 7.2 μ. Conidia green, globose to egg-shaped, spiny, 3.5 to 5.3 x 3.5 to 6.2 μ. Perithecia produced in abundance, yellow in color, spherical to elliptical, with an uneven surface, 85 to 150 μ in diameter; asci spherical to ovate, 8.8 to 14 μ in diameter, containing 5 to 8 smooth, elliptical spores, 4.8 to 5.6 x 5.6 to 6.4 μ (Pl. IV, fig. 3-6).

Hab. Isolated once from forest soil.

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Penicillium

This genus forms one of the largest groups of soil fungi; more species have been found in the soil belonging to this genus than to any other one. Some forms have been often found to form 2 to 3 colonies on a plate of 20,000 to 200,000 dilution. Very often plates of 20,000 dilution would contain 15 to 30 colonies of Penicillia, usually of one to four species. The writer has not been able to get many Penicillia from the direct soil inoculations, but from this it should not be concluded that these organisms are merely temporary visitors in the soil, and do not grow there and produce spores. If that should be the case, one would hardly expect to get Penicillia spores at a depth of 20 to 30 inches, because they would not be carried down by the water so easily, since they would germinate in the process of being carried down. The large numbers of spores found and the fact that they are found in all soils examined, cultivated and uncultivated, justify also the conclusion that the Penicillia are not merely brought in temporarily by some outside agency, but probably live in the soil. Oudemans (80) found 5 species of Penicillia in the soil, Jensen (53) also found 5, Dale (27) found 17 species of Penicillia and 3 species of Scopulariopsis in all the different types of soil that she has examined. The writer isolated about 35 well defined species and groups. Only about 25 of them are described here. A great deal of assistance in the identification of the Penicillia has been given to the writer by Dr. Charles Thom. Drawings and more complete description of the unidentified forms will appear later.

Luteum-purpurogenum group Thom

This is a typical group of soil organisms; more forms were found in the soil belonging to this group than to any other one; representatives have been isolated from all soils at one time or another. Several well-defined species belonging to this group have been isolated: *Penicillium luteum* Tukal, from the garden soil; *Penicillium pinophilum* (Hedgcock) Thom, forest soil; *Penicillium lilacinum* Thom, meadow soil; and *Penicillium purpurogenum*, var. *rubri sclerotium* Thom, meadow soil. Besides these, several forms have been isolated, belonging to this group, but which could not be placed with any well-described species; most of them were nearer the "luteum" end of the group. The differences were due only to the shade and change in color.

Penicillium chrysogenum Thom

Corresponds closely with Thom's description. One strain was isolated, which proved to be the same as Thom's No. 57; it produces an orange color on the reverse without coloring the medium.

Hab. Isolated from timothy, Sassafras, and California soils.

Penicillium commune Thom

Agrees closely to culture received from Dr. Thom.

Hab. Isolated once from orchard soil.

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Penicillium decumbans Thom

Hab. Isolated from forest soil.

Penicillium digitatum Sacc.

Hab. Isolated from garden, forest, and iron soils.

Penicillium expansum (Link) Thom

Hab. Isolated from forest soil.

Penicillium italicum Wehmer

Hab. Isolated from California soil.

Penicillium oxalicum Thom (A. 44)

Hab. Isolated once from garden soil.

Penicillium notatum Westling (C. 2)

Colony at first white, subfloccose, later becoming green, with a yellow reverse. Gelatin is liquefied in 7 to 8 days. Calcium oxalate crystals are formed abundantly on raisin agar. Conidiophores arising from the substratum, or as side branches of the aerial mycelium, smooth, up to 800 μ long and 2.8 to 5 μ thick, branching. Metulae 3 to 4.5 x 9.2 to 14 μ; sterigmata 2.4 to 3 x 7 to 8.4 μ. Conidia spherical, smooth, 2.6 to 3.3 μ in diameter. Closely agrees with culture received from Dr. Thom.

Hab. Isolated from garden, meadow, and forest soils.

Penicillium viridicatum Westling (A. 9)

Colony at first light green, slightly elevated above the substratum, restricted in growth; reverse yellow to brown, with the production of a yellow-brown pigment in the substratum. When old, surface becomes deep dull yellow-green (Rdg. xxxii, 31"-k) (88), granular, with the exudation of yellow drops of water upon the surface. Conidiophores, 50 to 500 μ long by 4 to 6 μ thick, arising as side branches of the aerial hyphae, verticillately branched, and ending in loose columns of spores; metulae 4 to 6 x 10 to 14 μ; sterigmata 3 to 3.5 x 8 to 9.5 μ. Conidia smooth, hyaline, globose, 2.6 to 4.4 x 3.3 to 4.4 μ. Liquefaction of the gelatin is rapid, starting at 5 days, at 18° C.

Hab. Isolated from garden, orchard, and iron soils.

Penicillium atramentosum Thom

Hab. Isolated from garden soil.

Penicillium rugulosum Thom

Hab. Isolated from forest soil.

Penicillium cyclopium Westling (D. 33)

Hab. Isolated once from forest soil.

Penicillium lividum Westling (D. 5)

Colonies at first white, with a green center and spreading margin, turning later gray-green and finaly becoming light Danube green (Rdg. xxxii, 35" k), with a white margin. Reverse yellow to yellow-brown, with the production of a soluble yellow pigment. No liquefaction of the gelatin in 15 days. Conidiophores arising

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as side branches of the aerial hyphae, 42 to 325 μ long by 2.4 to 3.6 μ thick, unbranched, ending in a verticillate head; sterigmata numerous, 2 to 2.5 x 8 to 12 μ. The verticills bear long chains of conidia, 120 to 750 μ long and forming close columns. Conidia greenish, elliptical to egg-shaped, or globose, granular, 3 to 4.2 x 3.4 to 4.8 μ. This organism has been identified by Dr. Thom.

Hab. Isolated from forest soil.

Penicillium glaber Wehmer (A. 16)

This organism belongs to the "Citromyces" group, formed by Wehmer. Colony is dark green in color, with a velvety surface; reverse at first ivory, later becoming dark to almost black; medicum gets a dark tinge. Vegetative hyphae 2.1 to 4.4 μ thick; conidiophores upright, septate, each ending in a single verticil of sterigmata. Conidia form long chains, the dense columns being 160 to 180 (80 to 830) μ long. Conidia uniform in size, globose, vacuolate, hyaline singly, green in mass, 2.4 to 3.2 μ in diameter. Sterigmata numerous, 2.4 to 3.2 x 8.8 to 10.5 μ. Gelatin liquefied in 8 days. Identified by Dr. Thom (Pl. IV, fig. 9).

Hab. Isolated many times from garden, orchard, forest, and Oregon soils.

Penicillium Pfefferianus Wehmer (D. 7)

This organism also belongs to the "Citromyces" group of Wehmer. Colony floccose, gray-green, with a whitish-gray tinge. Conidiophores arising as short branches of the aerial hyphae, 17 to 40 μ long by 3 μ thick, ending in a swelling, 4 to 7 μ in diameter, on which the sterigmata are borne; these are 2.6 to 3.5 x 6.2 to 8.8 μ. Conidia smooth, colorless to yellowish in mass, spherical, 2.4 to 2.8 μ in diameter; chains not broken when mounted.

Hab. Isolated from forest and Oregon soils.

Penicillium group I (13-25)

Colonies at first bluish gray-green (Rdg. xlii, 41'''), subfloccose, becoming with age brownish-green. Reverse at first colorless, later becoming hydrangea-pink (Rdg. xxvii, 5'' f), till finally it is red-brown; medium not discolored. Conidiophores usually arising as side branches of the aerial hyphae, septate, 65 to 330 μ long by 3.6 to 4.8 μ thick. Fructifications form loose divergent brooms, 40 to 250 μ long, with 1 or 2 divergent branches, sometimes none at all. Metulae 2.5 to 3.6 x 12 to 13.2 μ, each one bearing 2 to 3 sterigmata, 2.6 to 4.4 x 7.2 to 12 μ. Conidia globose to elliptical, vacuolate, smooth when young, granular when old, 2.6 to 3.5 x 2.6 to 4.4 μ. Gelatin is slowly liquefied, beginning after 14 days.

Hab. Isolated repeatedly from all the soils examined; very common in the soil.

Penicillium group II (6-22-23)

Colonies bright green with a brownish reverse; in older cultures surface turns gray-green to a dark ashy color; reverse becomes red-brown to dark yellow-brown; surface velvety. Conidiophores arise from the substratum or as short side branches of the aerial hyphae, 88 to 200 μ long by 1.8 to 2.6 μ thick, each dividing into several branches; each branch ends in a verticil of 3 to 6 sterigmata, bearing more or less divergent chains. Conidiophores variable in length; sterigmata 2.7 to 3.5 x 7 to 13.2 μ, bearing chains of spores 7 to 35 μ long. Conidia spherical, hyaline when young, brownish when old, thick-walled and spiny, 2.7 to 3.5 μ in diameter. Under certain conditions of moisture, the spores may be massed together into a ball, the chains instead of remaining distinct break down and form a gliocladium. Gelatin does not begin to liquefy until 4 weeks old. This organism greatly resembles Miss Dale's (C. 3).

Hab. Isolated from meadow, forest, and Oregon soils.

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Penicillium group III (9-24)

Colony floccose, at first white, with the production of a pink color in the reverse, later becoming gray to dirty yellow, with a deep red color in reverse, substratum becoming yellowish. On raisin agar the colony is sulphur-yellow with no pink pigment produced. Vegetative hyphae creeping, branched, septate, 2 to 3 μ in diameter, often forming ropes of hyphae. Conidiophores arising alternately as side branches of aerial hyphae, 38 to 1000 μ long by 2.7 to 3.5 μ thick, septate; the nodes are characteristically swollen up to 5 μ in thickness. Conidiophores forming 2 to 3 metulae, pointed at both ends, 3.5 to 4.6 x 10 to 25 μ; each metula bears few sterigmata, 2.3 to 2.7 x 7.2 to 13.2 μ. Chains of spores are divergent, 12 to 35 μ long, of unequal size, with no close broom formation. Chains of spores unbroken when mounted; conidia smooth, vacuolate, globose and elliptical, often pointed at one end; 2.7 to 3.0 x 2.6 to 3.5 μ. No liquefaction of the gelatin.

Hab. Isolated from meadow and forest soils.

Penicillium group IV (2-11-15)

Colonies floccose, white to gray, with an intermixture of yellow; when old the color becomes darker, with a violet tinge; reverse colorless, then creamy, finally dark. Conidiophores arising as alternate side branches of the vegetative hyphae, 25 to 85 μ long by 3 μ thick, septate, unbranched, ending in a verticil of 2 to 3 metulae, 4 x 15 to 22 μ; each metula carries 1 to 3 sterigmata, 3.5 to 5.2 x 5.2 to 12 μ. Conidia form short chains, unbroken when mounted, globose to somewhat ellipsoidal, 2 to 2.6 x 2.2 to 3.4 μ. Liquefaction of the gelatin starting only in 14 days and advancing very slowly.

Hab. Isolated from orchard, timothy and Sassafras loam soils.

Penicillium group V (18-20)

Colony at first pale blue (Rdg. viii, 45 f), later changing to greenish-glaucous-blue (Rdg. xlii, 41''' C); reverse is yellow-brown to ivory, slightly coloring the medium yellow. Surface of colony velvety. Conidiophores either terminal or arising as side branches of the aerial hyphae, 85 to 215 μ long by 2.7 to 3.5 μ thick. Conidial fructifications, up to 250 μ long, consist of simple verticils of sterigmata, bearing divergent chains of spores. Metulae 3 x 8.8 to 15.2 μ; sterigmata 2.4 to 3.2 x 7.9 to 10.2 μ. Conidia globose, hyaline, smooth, 2.7 to 3.6 μ in diameter. Liquefaction of the gelatin is very rapid, starting at 5 days, and is almost complete in 10 days.

Hab. Isolated from orchard and forest soils.

Penicillium group VI (10)

Colony at first pale green to dull glaucous-blue (Rdg. xlii, 41''' f), later becoming chocolate-brown; reverse creamy, medium clear; surface velvety. Conidiophores arising directly from substratum, 330 to 500 μ long by 2 to 3.5 μ thick, and ending in a verticil of sterigmata, carrying a side branch or none at all. Metulae 2.8 x 5.3 to 13.2 μ, sterigmata 2.8 x 8.4 to 11 μ; chains of spores greatly divergent. Conidia spherical to elliptical, hyaline, smooth, 2.4 to 2.8 x 2.4 to 3 μ. Liquefaction of the gelatin is very slow, starting when culture is only 18 days old.

Hab. Isolated from garden and iron soils.

Penicillium desiscens Oud. (C. 15)

This organism resembles Jensen's description more closely than Oudeman's. Colony velvety, dark yellowish-green (Rdg. xviii, 33' m), constricted in growth; reverse pale yellow without coloring the substratum. Conidiophores hyaline, septate,

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165 to 600 μ high by 3.5 to 5.2 μ in diameter, very seldom forming side branches, but mostly branching verticillately. Conidiophores ending in a verticil of 3 to 6 metulae, 3.1 to 4.4 x 10 to 13.6 μ, each metula producing 4 to 6 sterigmata, 3.5 to 4.4 x 5.2 to 8.2 μ. Conidial fructification is constricted at the base, forming a more or less close broom, 80 to 500 μ long by 25 to 80 μ wide. Conidia light green, ellipsoidal, 2.2 to 2.8 x 2.6 to 3.5 μ (Pl. IV, fig. 10-12).

Hab. Isolated once from meadow soil.

Scopulariopsis

Scopulariopsis brevicaule Saccardo (n. var. ?) (A. 27)

Colonies gray, turning yellowish-brown to chocolate color; surface velvety, margin broadly spreading. Gelatin rapidly liquefied in 5 days with the production of a strong ammoniacal odor. Conidiophores short, 16 to 42 μ high by 4 to 4.4 μ thick, usually borne as lateral branches of the aerial hyphae, sparingly branching or forming simple chains of conidia. Sterigmata tapering at the end, 3.8 to 4.3 x 13 to 17.2 μ. Conidia light brown in mass, globose to somewhat pear-shaped, with a thick spiny wall, 6.2 to 8.8 μ in diameter (Pl. IV, fig. 7, 8).

Hab. Isolated from garden and iron soils.

Cephalosporium

Cephalosporium acremonium Corda (C. 51)

Colonies snow-white, floccose, with a rose-colored center; later all the colony becomes rose-colored, except the white floccose margin. Vegetative hyphae hyaline, fine, creeping, sparsely septate, branched, 2.2 to 3.1 μ thick. Conidiophores arising as side branches of the vegetative hyphae, unseptate, unbranched, 2.3 to 3.1 x 18 to 44 μ, bearing at the tip a head of conidia; the head is globose, 13 to 20 μ in diameter; conidia numerous, oblong to cylindrical, 1.5 to 2.1 x 4 to 6.6 μ, rose-colored.

Hab. Isolated several times from meadow soil; also by Koning in Holland.

Cephalosporium curtipes (?) Saccardo (C. 35)

Colony orbicular, floccose, white. Vegetative hyphae creeping, branched, septate, hyaline, filled with oil globules, 8.8 to 13.2 μ in diameter. Conidiophores erect, short, unbranched, unseptate, arising as side branches of the vegetative hyphae, 3 to 7 μ long; conidial head globose, 7 to 9.5 μ in diameter; conidia egg-shaped to oblong, filled with minute oil globules, 2.3 to 4 x 4.8 to 13.2 μ.

Hab. Isolated once from meadow soil.

Cephalosporium sp. (G. 23)

Colony floccose, white, later becoming yellowish in color. Mycelium hyaline, creeping, branching dichotomously, sparsely septate, 3.5 to 4.2 μ in diameter; conidiophores arising as side branches of the vegetative hyphae, but are not swollen very much at the tip, 1.8 to 2.5 x 26.4 to 35.2 μ; conidia arising singly at the tip of the conidiophore, pressed to the side by those produced next, they adhere to one another by means of a slime, resulting finally in a head of conidia; heads spherical, greatly variable in size, 17 to 100 μ in diameter, breaking off easily; conidia egg-shaped to elliptical, smooth, granular contents, 2.8 to 4.4 x 5.3 to 9.4 μ (Pl. V, fig. 1, 2).

Hab. Isolated once from iron soil.

Cephalosporium sp. (D. 32)

Colonies dense floccose, white, later becoming green to gray-green, with a white woolly margin. Vegetative hyphae hyaline, creeping, much septate, branched,

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4.4 to 7.2 μ in diameter; mycelium has a tendency to form ropes of intermingled hyphae. Conidiophores arising as short side branches of the vegetative hyphae, unseptate, unbranched, pointed at the upper end, 3.8 to 6.4 x 13 to 18 μ; conidia elliptical, bright green singly, of a deeper green in mass, 2.8 to 3.6 x 3.5 to 4.8 μ, borne singly, but united together to form a head, 14 to 18 μ in diameter; the head is either borne on the short conidiophores, or directly upon the main hyphae. This species may be closely allied to the Trichodermae, but by the character of the conidiophores and the head it is closely related to the Cephalosporia (Pl. IV, fig. 15).

Hab. Isolated from forest soil.

Cephalosporium n. sp. (?) (C. 56)

Colony thin, white, with rosy conidial masses; growth is hardly noticeable with the naked eye, except when the rosy masses of conidia appear. Hyphae hyaline, septate, creeping, branched, 4.4 μ thick; conidiophores arising as side branches of the vegetative hyphae, somewhat thinner than these, 170 to 350 μ long, branching, often with secondary branches, each branch slightly pointed at the tip and carrying a head of conidia; head 8.5 to 17.5 μ in diameter; conidia colorless, egg-shaped, cylindrical to bean-shaped, greatly varying in size, 2.7 to 3.5 x 4.4 to 8.8 μ (Pl. V, fig. 3-5).

Hab. Directly isolated from the meadow soil.

Trichoderma

This genus, of which only very few species are fully described, occurs commonly in the soil. The two known green forms, *Trichoderma Koningi* and *Trichoderma lignorum*, either one or both of them together, have been isolated repeatedly from the soil by most investigators on soil fungi, from Oudemans (80) to Dale (27). Cook and Taubenhaus (23) have also shown that these organisms cause storage rot of sweet potatoes. The green Trichoderma have been found at one time or another in all the soils examined, especially in the meadow and forest soils, from which these organisms have been isolated repeatedly. In general 5 strains of green Trichoderma have been isolated by the author.

Strain I (C. 16). Colony white, floccose, spreading; green masses begin to appear only when culture is 7 to 8 days old, forming isolated groups, without any zonation. Vegetative hyphae hyaline, septate, branched; conidiophores arising as side branches on the mycelium, septate and branched mostly opposite, each branch carrying a conidial head, 8 to 12 μ in diameter; spores elliptical, 2.2 to 3 x 3.1 to 4.4 μ, hyaline to rare green; chlamydospores abundant, 6.2 x 8 μ. This organism coincides more closely with *Trichoderma Koningi* Oud. than any other one.

Hab. Isolated repeatedly from meadow, forest, iron, and Oregon soils.

Strain II (D. 16). Colony at first white, with fine, hyaline, spreading mycelium. Green conidial masses begin to appear at the center, spreading gradually toward the periphery with the formation of green concentric zones; the zonation is characteristic of this group. Vegetative hyphate creeping, septate; conidiophores do not differ very much from those of the previous organism: there is less septation and greater variation in the size of the conidial head, which is 7 to 17.5 μ in diameter. Conidia globose, green, 3.2 to 3.5 μ in diameter; no chlamydospores were observed. This organism is closely related to *Trichoderma lignorum* (Tode) Harz.

Hab. Isolated repeatedly from meadow and forest soils.

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Strain III (G. 5). Colonies at first colorless, thin, rapidly spreading, nonfloccose; later becoming light green and changing into a dark green color, with no conidial tufts, as in Strain I, and no zonation as in Strain II. Entire colony soon becomes solid green. Vegetative hyphae hyaline, creeping, septate, and branched. Conidiophores with little branching and carrying fairly large heads of conidia, 17.5 to 35 μ in diameter. Conidia light green, spherical to elliptical, somewhat pointed at one end, 3.5 to 4.4 x 4.4 to 6 μ, largest of all the groups. Chlamydospores abundant, spherical, 8.8 to 13.2 μ in diameter.

Hab. Isolated from iron, garden and forest soils.

Strain IV (C. 10). Colonies at first white, floccose, spreading, with green tufts appearing all over the plate and soon covering the whole surface; the green tufts are more abundant than the vegetative growth, while Strain I had just the opposite relation. Conidiophores arising as side branches of the vegetative hyphae, branched, septate; conidial heads 7 to 10 μ in diameter. Conidia light green, usually elliptical to egg-shaped, but many globose spores are found; conidia 2.7 to 3.2 x 3.1 to 3.6 μ. The large size of the head and the peculiar method of its formation differentiates this organism from the other Trichodemae.

Hab. Isolated from meadow and forest soils.

Strain V (D. 34). Colony very thin, transparent, spreading, with no aerial mycelium except at the very margin. Conidial masses large, with no separate tuft formation, soon covering the whole surface. Vegetative hyphae creeping in substratum, branched, septate. Conidiophores branching, each one ending in a conidial head, 7 to 12 μ in diameter. Conidia elliptical to almost cylindrical, 2.5 to 2.9 x 3 to 3.5 μ, light green singly, dark green in mass; chlamydospores abundant, 5.2 to 8.8 μ in diameter.

Hab. Isolated from forest soil.

Trichoderma album Preuss (C. 17)

Colony thin, white, spreading; small aerial white tufts, 1 to 5 mm. in diameter, appearing on the surface of the colony, without forming regular zones and without covering the whole surface. Vegetative hyphae hyaline, branched, septate; conidiophores arising as side branches of the aerial mycelium, 4 to 4.6 μ in diameter, septate, branching, with small secondary branches; conidial heads 7.5 to 14 μ in diameter; conidia hyaline, elliptical, 1.8 to 2.1 x 2.5 to 2.9 μ.

Hab. Isolated several times from meadow and forest soils.

Acrostalogmus

Acrostalogmus cinnabarimus var *nana* Oud. (A. 3)

The general characters and description of this organism coincide very closely with those given by Oudemans (80).

Hab. Isolated repeatedly from garden, orchard, meadow, and Oregon soils; also by Koning in Holland and Goddard in Michigan.

Acrostalogmus albus Preuss.

Colonies white, thin, subfloccose, spreading rapidly. Vegetative hyphae hyaline, creeping, little branched, septate. Conidiophores arising as side branches of the vegetative hyphae, erect or ascending, up to 200 μ long by 1.5 to 2.1 μ thick, branching at the tip, forming 1 to 6 branches; branches alternate, slightly curved and pointed at the tip. A cap of conidia, 9 to 12 μ in diameter, is borne at the tip of each branch; conidia hyaline, long ellipsoidal, 1.1 to 1.5 x 2.9 to 3.7 μ.

Hab. Isolated from garden and meadow soils.

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Verticillium

Verticillium glaucum (?) Bonorden

Colony white, later becoming green from the formation of spores; old cultures are all green, with a yellowish tinge. Mycelium creeping, 3 to 6 μ in diameter; sterigmata arising from conidiophore either singly or in whorls, slightly swollen at the base and pointed at the tip, 3 to 5 x 6 to 10 μ; chlamydospores present; conidia hyaline, spherical, 2.5 to 3 μ in diameter.

Hab. Isolated from forest soil.

Verticillium terrestre (?) (Link) Lindau (G. 16)

Colony snow-white, floccose, spreading, with the production of rose-colored powdery conidial masses. Vegetative hyphae creeping, branched, septate, hyaline; conidiophores upright, septate, branched, with a well-pronounced main stem, 4 to 6.4 μ thick, with four branch whorls; conidia formed singly at the tip of the branches, soon falling off, hyaline, globose to slightly elliptical, 3.8 to 4.5 x 4.4 to 4.8 μ.

Hab. Isolated from forest and iron soils.

Cephalothecium

Cephalothecium roseum Corda

Colony at first white, with a rose tinge, later becoming rose to almost red; growth powdery, spreading, of a cobweb-like nature; vegetative hyphae creeping, septate, branched. Conidiophores unseptate, unbranched, 3 to 3.8 x 200 to 500 μ; conidia produced at the tip of the conidiophore singly, one after another, forming a loose head; conidia pear-shaped, two-celled, rose-colored, 7.6 to 12.4 x 11.4 to 20 μ.

Hab. Isolated from meadow soil; also by Jensen in Ithaca.

Dicoccum

Dicoccum asperum Corda (A. 34)

Colony white, floccose, becoming dark to almost black with a shade of yellow; reverse yellowish black. Mycelium consists of hyaline, branched, sparely septate, yellowish hyphae, 2.6 to 3.5 μ thick; conidiophores arising as short side branches, 3 to 12 μ long. Conidia at first hyaline, then brown to black, oval, two-celled; upper cell spherical, brown, thick-walled, spiny; lower cell usually smaller, spiny; the whole spore is 20 to 22 μ long, 9 to 13 μ thick.

Hab. Isolated from garden soil.

Zygodesmus

Zygodesmus sp. (B. 36)

Growth floccose, gray, up to 2 cm. high; vegetative hyphae branched, septate, 7 to 10 μ thick. Spores are borne around the tip of the side branches, which vary in length, often almost lacking; the tip of the branch is either not differentiated from the rest or swollen to 27 μ in thickness. The size of the spores varies with the strains; the one isolated from the orchard soil has smooth, yellow, granular spores, spherical to elliptical or egg-shaped, 8.8 to 17.6 x 15 to 22 μ; the Oregon strain has spores of the same shape and color, but smaller in size, being only 5.4 to 8.8 x 6.2 to 10.4 μ; wall either smooth or spiny (Pl. V, fig. 6-9).

Hab. Isolated from orchard and Oregon soils.

Dematiaceae

Basisporum gallarum (?) Molliard (G. 8)

Colony at first white floccose, later becoming dark brown to almost black, with a brownish yellow to black reverse. Mycelium hyaline, branched, septate, creeping,

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bearing along its hyphae globose brown to black spores; hyphae filled with oil globules; spores are borne on short lateral branches or directly upon the hyphae; the branches are 4 to 6 x 8 to 12 μ, some of which may consist of two cells; spores spherical, 12 to 15 μ in diameter.

Hab. Isolated from iron soil.

Cladosporium herbarum (Pers.) Link (C. 26)

Syn.: *Hormodendrum cladosporoides* (Fres.) Sacc.

Colony at first white, turning gray to gray-brown, subfloccose; reverse black. Mycelium branched, septate, 2 to 4.6 μ thick; conidiophores much branched, septate, olivaceous in color, 4 to 8.8 x 100 to 200 μ; conidial tufts borne at the end of all the branches. Spores are produced in great abundance, varying greatly in size and shape, always olivaceous, slightly elongated and unicellular, 2.2 to 3.8 x 2.8 to 6 μ.

Hab. Isolated repeatedly from garden, orchard, meadow, forest and Oregon soils; also by Jensen in Ithaca, Dale in England, and Goddard in Michigan.

Cladosporium epiphyllum Pers.

Colony at first white, then greenish, and finally gray rusty-brown on the surface and black on the reverse. Conidial masses are borne as side branches of the long radiating hyphae; conidia very numerous, round to cylindrical, at first unicellular, then often with one septum, olive green, 3.8 to 5.2 x 10 to 14 μ (Pl. V. fig. 13).

Hab. Isolated from garden, meadow and California soils; also by Dale in England.

Dematium pollulans (?) de Bary (N. 42)

Colony velvety, dark brown to almost black, reverse black. Hyphae branched, closely septate, brown, 2.8 to 6.2 μ thick. Spores spherical to elliptical, brown, smooth, 3 to 4.4 x 3 to 5.2 μ.

Hab. Isolated once from California soil.

Acremoniella sp. (?) (C. 37)

Colony floccose, spreading, at first white, later turning dark to almost black, with black reverse, aerial mycelium remaining gray. Hyphae creeping, branched, hyaline, with very short side branches, carrying single spores at the tip; spores spherical to egg-shaped, black, 5.2 to 8.8 x 6.2 to 8.8 μ.

Hab. Isolated once from meadow soil.

Alternaria humicola (?) Oudemans (C. 8)

This culture agrees closely with the description given by Oudemans (80).

Hab. Isolated from meadow soil; also by Koning in Holland.

Alternaria sp. (A. 36)

Mycelium branching, hyaline, 3.5 μ thick. Branching chains of spores arising as side branches of the vegetative hyphae. Spores 1 to 4 septate with no cross septa, not or only slightly constricted at the place of septation; spores from almost spherical to cylindrical, 8 to 15 x 15 to 40 μ.

Hab. Isolated from garden and meadow soils.

Alternaria sp. (B. 20)

Colony floccose, dark brown, black on reverse; aerial mycelium gray with a greenish tinge, up to 2 cm. high. Conidia occurring in short chains of 1 to 4 spores;

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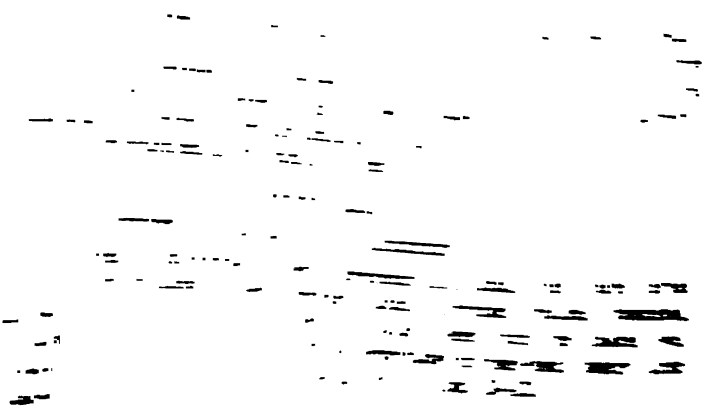

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Sclerotium Tode

Several types of this organism have been isolated from the different soils. Vegetative hyphae usually colorless, creeping, branched; sclerotia spherical or elongated, singly or in masses, usually black, hard when dry, with a distinct cell division.

Sterile white mycelium

Snow-white sterile mycelium isolated repeatedly from all soils; growth floccose; hyphae hyaline, septate, branching. No conidial structures ever found; some types were found to form chlamydospores.

Sterile red mycelium

Hab. Isolated from garden, forest and Oregon soils.

Summary of Morphological Studies

Altogether there were isolated from the soil over one hundred distinct species of fungi, belonging to 31 genera. Some of the organisms have been grouped together, because the different types either did not show enough distinctive characters, or were not thought important enough to be separated into different species. There were several organisms left not included in the present description, due to the difficulty of their identification. The writer tried to avoid as much as possible the making of new species, leaving it to the special students on fungi.

The following genera of fungi were found to be represented in the soil:

1. Absidia
2. Mucor
3. Rhizopus
4. Zygorhynchus
5. Saccharomyces
6. Chaetomium
7. Monilia
8. Oidium
9. Sporotrichum
10. Botrytis
11. Aspergillus
12. Penicillium
13. Scopulariopsis
14. Verticillium
15. Cephalosporium
16. Trichoderma
17. Acrostalagmus
18. Zygodesmus
19. Dicoccum
20. Cephalothecium
21. Basisporium
22. Dematium
23. Acremoniella
24. Cladosporium
25. Alternaria
26. Fusarium
27. Melanconium
28. Coniothyrium
29. Sclerotium
30. Sterile white mycelium
31 Sterile red mycelium

The most common genera of fungi, as to numbers and species, found in the soils investigated are, in the order of their occurrence, as follows: Penicillium, Mucor, Aspergillus, Trichoderma, Cladosporium, Fusarium,

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Cephalosporium, Rhizopus, Zygorhynchus, Acrostalagmus, Alternaria, and Verticillium. Going back to the work of other investigators on soil fungi, these genera are found, with one or two exceptions, also isolated by Jensen (53) and Dale (27). Hagem (43) mentions the Penicillia, Aspergilli and Mucors, as the three largest groups of soil fungi.

TABLE III

ISOLATION OF COMMON GENERA OF SOIL FUNGI BY DIFFERENT INVESTIGATORS

Genus	Koning	Dale	Jensen	Goddard	McLean and Wilson	Author
Acrostalagmus	*	..	*	*	..	*
Alternaria	*	*	*	..	*	*
Aspergillus	*	*	*	*	*	*
Cephalosporium	*	..	..	..	..	*
Cladosporium	..	*	*	*	*	*
Fusarium	..	*	..	*	..	*
Mucor	*	*	*	*	*	*
Penicillium	*	*	*	*	*	*
Rhizopus	..	*	*	*	*	*
Trichoderma	*	*	*	*	*	*
Verticillium	..	*	..	*	..	*
Zygorhynchus	..	*	*	..	*	*

As seen from the results as expressed in Table III, the genera Aspergillus, Mucor, Penicillium, and Trichoderma have been isolated by all six investigators of soil fungi, and hence represent a flora studied at different parts of the world. The other genera are reported by two or more investigators. These 12 genera are all reported by the author, as isolated from most soils studied; Koning (80) found 7 out of these 12, Dale (27) 10, Jensen (53) 9, Goddard (41) 9, and McLean and Wilson (73) 8. Altogether over 50 genera of fungi have been reported to have been isolated from the soil; most of then having been isolated in one, two, or more cases. Not enough work has yet been done on the subject to enable one to say with certainty that these are the soil genera; but as far as the work done shows, the Aspergilli, Mucors, Penicillia, and Trichodermae seem to be found in all soils.

As to particular species of soil fungi found in the different soils studied, it is not so easy to decide which species are actually soil forms and are found in all the soils; first, because the environmental conditions are so different that some forms found in one locality, under one particular set of conditions, may not be found in another; secondly, the different investigators might differ in their opinion as to the identification of a particular species, especially since some of them are very variable, and the methods of study, such as media, incubation, etc., are not standardized. For that reason, it is better to speak of groups of organisms as soil

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forms, rather than as individual species. But some species are found to be reported by many investigators, as having been isolated from the soil at different parts of the world. For example, *Trichoderma Koningi, Cladosporium herbarum, Mucor racemosus,* and *Rhizopus nigricans* have been reported from different parts of the world. Several species of Mucors have been isolated by Hagem (42) in Norway, by Dale (27) in England, and by Jensen (53) and the author in this country.

The question then arises: "Is there any so-called fungus flora of the soil?" The data at hand do not as yet warrant any positive conclusion. But considering the fact that there are groups of soil fungi which were found in all, or at least, most soils studied, whether acid, neutral, or alkaline, whether cultivated or not, under different climatic conditions, one might conclude that if not all forms, or even most of them, there are some fungi which are always found in the soil, and these are soil fungi proper.

The fact that the genera Aspergillus, Mucor, Penicillium, and Trichoderma were found in all the investigations, where a complete survey of the soil flora was made, leads one to think that these organisms, associated with others, make up the fungus flora of the soil. A hypothetical soil flora should then consist of several species of Aspergilli, more than those in types (not always in numbers) of Mucors and Penicillia, together with the green Trichodermae; besides these, one would expect to find a Zygorhynchus, one or two species of Cladosporia, Alternaria, Rhizopus, Fusaria, Verticillia, Cephalosporia, associated perhaps with an Acrostalagmus, a Scopulariopsis, a Botrytis, some Sterile Mycelium, and one or two Yeasts. This would probably form the average fungus flora, to which of course should be added organisms found only in one particular soil and not found in another.

Of course, attention might be called to the fact that all the forms ascribed to one investigator have been isolated by him, not from one soil, but from several soil types; this fact is due to the taking of the samples only in one spot, at one time, and in one season of the year. Had the samples been taken repeatedly and from different parts of the field, the other forms also might have been isolated. It is the author's belief that the larger the number of samples taken from one soil for study, the nearer the fungus flora found would approach the average flora outlined above. Of course, the species should be studied, not the genera alone, because only the species found can give a true basis for comparison, but the stress should be laid, not only on the occurrence of the particular species, however important this might be, but also on the occurrence of the group of which the species is a representative. This will help us to get a clear insight into the flora of the soil.

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PHYSIOLOGICAL STUDIES ON SOIL FUNGI

A. Nitrogen Fixation

Mannite solution was made up according to the following formula (70):

Distilled water	1000 c.c.
K_2HPO_4	0.20 gm.
$MgSO_4$	0.20 gm.
$CaCl_2$	0.02 gm.
$FeCl_3$	Trace
Mannite	15.00 gm.

This was distributed into 250-c.c. Erlenmeyer flasks, 100 c.c. of the solution in each flask. After the flasks were sterilized, they were inoculated with the desired organisms and allowed to incubate for 30 days at 25° C. The contents of the flasks were then transferred to Kjeldahl flasks and the regular Kjeldahl determinations made. The results are given in Table IV.

TABLE IV

NITROGEN FIXED PER 100 C.C. MANNITE SOLUTION

Name of Organism	Total mg. N.	Duplicate determination mg. N.	Average mg. N.	Minus check mg. N.
Penicillium luteum	.735	1.029	.882	0.280
Penicillium sp.	.735	.735	.735	0.133
Alternaria sp.	.588	.882	.735	0.133
Rhizopus nigricans	.882	.735	.809	0.207
Cephalothecium roseum	.588	.441	.515	—0.087
Check	.616	.588	.602	.....

The amount of nitrogen fixed lies within the analytical error and is negligible. The results confirm the investigations of Goddard (41) and others, who could not prove any nitrogen fixation by fungi.

B. Ammonification

For the determination of the ammonifying power of fungi the following method was used: 100 gm. of sieved air-dry soil of a light loamy texture were placed in 250-c.c. Erlenmeyer flasks; 155 mg. of nitrogen in the form of dried blood and cottonseed meal were added to the soil and well mixed in by shaking the flask thoroughly; 20 c.c. of water were then added to the flask, this amount forming about 70 per cent of the moisture-holding capacity of the soil. The flasks were then numbered, plugged with cotton, and sterilized in the autoclave at 15 pounds pressure for 15 minutes. The organisms used for inoculation were grown for 6 to 10 days on the No. II liquid medium, or Czapek's solution. The liquid culture of the organism was well shaken, and 1 c.c. of the spore-containing liquid was used for inoculation. The flasks were then incu-

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bated at 22° C. for 12 days, since this period was found by the author (115) to be the most favorable one for the comparison of the ammonifying power of the different organisms. After that period the soil was transferred with enough water into copper flasks, and distilled over with MgO into standard HCl solution.

TABLE V

AMMONIA ACCUMULATION BY FUNGI IN 12 DAYS

Name of Organism	Dried Blood as source of N.				Cottonseed Meal as source of N.			
	Ammonia N. found. mg. N.		Average	Increase over check	Ammonia N. found. mg. N.		Average	Increase over check
Mucor racemosus	20.24	16.98	18.61	15.91	50.12	54.48	52.30	48.10
Mucor plumbeus	12.42	15.36	13.89	11.19	47.72	40.50	44.11	39.91
Mucor hiemalis I	20.42	16.80	18.61	15.91	38.97	36.17	37.57	33.37
Mucor hiemalis II	18.00	16.28	17.14	14.44	40.14	33.50	36.82	32.63
Rhizopus sp. I (B. 2)	23.35	21.15	22.25	19.55	47.11	51.47	49.29	45.09
Rhizopus sp. II	20.76	20.36	20.56	17.86	50.68	46.24	48.46	44.26
Rhizopus sp. (B. 5)	33.20	30.02	31.62	28.92	52.69	46.69	49.69	45.49
Rhizopus sp. (B. 3)	16.46	14.16	15.31	12.61	43.74	43.74	43.74	39.54
Rhizopus sp. (C. 5)	40.16	38.00	39.08	36.38	56.75	52.11	54.43	50.23
Zygorhynchus Vuilleminii	12.54	10.18	11.36	8.66	47.58	45.18	46.38	42.18
Aspergillus sp. (B. 16)	21.20	24.20	22.70	20.00	31.60	33.64	32.62	28.42
Penicillium luteum	4.59	4.99	4.79	2.09	14.40	14.14	14.27	10.07
Penicillium lilacinum	25.12	27.20	26.16	23.46	28.12	26.00	27.06	22.86
Penicillium chrysogenum	23.00	25.02	24.01	21.31	23.13	25.33	24.23	20.03
Penicillium sp. (9-24)	17.28	16.20	16.74	14.24	37.18	39.54	38.36	34.16
Penicillium decumbens	3.89	3.59	3.69	0.99	16.71	17.11	16.91	12.71
Penicillium lividum	5.10	4.24	4.67	1.97	17.67	20.07	18.87	14.67
Penicillium sp. (D. 12)	15.41	15.41	15.41	12.71	24.12	26.52	25.32	21.12
Penicilium sp. (C. 7)	72.12	81.16	76.64	73.94	50.13	46.53	48.33	44.13
Penicillium Pfefferianus	3.72	3.96	3.84	1.14	10.92	9.24	10.08	5.88
Penicillium digitatum	6.00	7.20	6.60	3.90	31.60	30.20	30.90	26.70
Penicillium glaber	6.90	6.50	6.70	4.00	14.20	14.80	14.50	10.30
Penicillium italicum	8.10	8.30	8.20	5.50	25.40	27.00	26.20	22.00
Trichoderma Koningi	87.65	95.85	91.75	89.05	79.12	81.73	80.43	76.23
Monilia sitophila	40.12	42.24	41.18	38.48	58.21	61.60	59.91	55.71
Cephalothecium roseum	36.24	38.84	37.54	34.84	53.30	56.29	54.80	50.60
Cephalosporium sp. (B. 7)	39.15	39.75	39.45	36.75	50.12	52.72	51.42	47.22
Acrostalagmus cinnabarinus	40.20	44.20	42.20	39.50	72.60	74.72	73.66	69.46
Dematium pollulans	22.25	22.25	22.25	19.55	48.09	50.48	49.29	45.09
Alternaria humicola	53.04	61.13	57.09	54.39	64.02	68.46	66.24	62.04
Cladosporium herbarum	16.12	16.76	16.44	13.74	36.80	38.83	37.82	33.62
Fusarium caudatum	33.30	39.30	36.30	33.60	55.20	58.37	56.79	52.59
Bacterium mycoides	25.55	23.16	24.36	21.46	21.12	25.73	23.43	19.23
Bacillus proteus	10.00	10.10	10.05	7.35	11.52	13.71	12.62	8.42

Looking through Table V, one can readily see that all the fungi examined for their ability to decompose organic matter and liberate ammonia, do it quite readily. When the fungi are compared with the bacteria tested for their power to accumulate ammonia, many of them are found to be much stronger ammonifiers even than *Bacterium mycoides,* which is supposed to be a strong ammonifying organism. A great variation in

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TABLE VI

INFLUENCE OF AGE OF CULTURE UPON THE AMMONIFYING POWER OF FUNGI

Name of Organism	Young Cultures								Cultures Six Months Older							
	Dried Blood				Cottonseed Meal				Dried Blood				Cottonseed Meal			
	Ammonia N. found		Average mg. N.	Increase over check mg. N.	Ammonia N. found		Average mg. N.	Increase over check mg. N.	Ammonia N. found		Average mg. N.	Increase over check mg. N.	Ammonia N. found		Average mg. N.	Increase over check mg. N.
	mg. N.	mg. N.			mg. N.	mg. N.			mg. N.	mg. N.			mg. N.	mg. N.		
Mucor racemosus	20.24	16.98	18.61	15.91	50.12	54.48	52.30	48.10	48.00	45.20	46.60	43.30	69.60	76.30	72.95	68.25
Mucor plumbeus	12.42	15.36	13.89	11.19	47.72	40.50	44.11	39.91	27.00	29.10	28.05	24.75	36.00	38.20	37.10	32.40
Mucor hiemalis	20.42	16.80	18.61	15.91	38.97	36.17	37.57	33.37	24.10	26.70	25.40	22.10	38.20	36.30	37.25	32.65
Rhizopus nodosus	40.16	38.00	39.08	36.38	56.75	52.11	54.43	50.23	15.60	13.50	14.55	11.25	42.60	37.50	40.05	35.35
Zygorhynchus Vuilleminii...	12.54	10.18	11.36	8.66	47.58	45.18	46.38	42.18	22.50	28.30	25.40	22.10	66.10	61.70	63.90	59.20
Penicillium chrysogenum....	23.00	25.02	24.01	21.31	23.13	25.33	24.23	20.03	11.20	7.60	9.40	6.10	27.00	26.20	26.60	21.95
Penicillium luteum	4.59	4.99	4.79	2.09	14.40	14.14	14.27	10.07	4.80	4.20	4.50	1.70	3.20	3.60	3.40	—1.00
Penicillium lilacinum	25.12	27.20	26.16	23.46	28.12	26.00	27.06	22.86	7.50	7.70	7.60	4.80	9.00	10.40	9.70	5.30
Penicillium sp. (9-24)	17.28	16.20	16.74	14.24	37.18	39.54	38.36	34.16	7.80	6.20	7.00	4.20	10.40	9.20	9.80	5.40
Penicillium lividum	5.10	4.24	4.67	1.97	17.67	20.07	18.87	14.67	2.10	1.90	2.00	—0.80	10.60	11.20	10.90	6.50
Penicillium sp. (C. 7)	72.12	81.16	76.64	73.94	50.13	46.53	48.33	44.13	24.70	21.90	23.30	20.00	29.80	30.60	30.20	25.50
Cephalothecium roseum	36 24	38.84	37.54	34.84	53.30	56.29	54.80	50.60	30.40	25.20	27.80	24.50	51.20	54.40	52.80	48.10
Acrostalagmus cinnabarinus..	40.20	44.20	42.20	39.50	72.60	74.72	73.66	69.46	16.50	13.70	15.10	11.80	60.60	58.20	59.40	54.70
Dematium pollulans	22.25	22.25	22.25	19.55	48.09	50.48	49.29	45.09	10.60	12.02	11.31	8.01	38.12	42.43	40.28	35.58

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the ammonifying power is found between the different groups of organisms, the strongest one being *Trichoderma Koningi*, a very common soil form, liberating in 12 days, in the form of ammonia, 57.5 per cent of the nitrogen in the dried blood, and 49.2 per cent in the cottonseed meal. The Penicillia proved to be, as a rule, weak ammonifiers, with only one exception. The great variation between the different Penicillia in the ammonifying power is probably due to the fact that the genus is so large, and that there are combined many organisms only distantly related. For that reason such a variation could be found as between the *Penicillium decumbeus* liberating, in the form of ammonia, 0.6 per cent of the nitrogen in dried blood and 8.2 per cent of the nitrogen in cottonseed meal, and *Penicillium* sp. (C. 7) liberating, in the form of ammonia, 47.7 per cent of the nitrogen in dried blood and 28.5 per cent of the nitrogen in cottonseed meal, under the same environmental conditions and in the same period of time.

The ammonifying power of the Mucorales, including representatives of the genera Mucor, Rhizopus, and Zygorhynchus, show less variation than the Penicillia. The extremes of these groups represent *Rhizopus* sp. (C. 5), with the accumulation of nitrogen, in the form of ammonia, 23.5 per cent in the dried blood and 32.4 per cent in the cottonseed meal, and *Zygorynchus Vuilleminii* with 5.6 per cent in the dried blood, and *Mucor hiemalis* with 21 per cent in the cottonseed meal. All the other fungi tested gave fairly large accumulation of ammonia, including the Monilia, Alternaria, Fusaria, Cephalosporia, and the others.

Some of the organisms, having been kept in culture on artificial media for six months, were tested again for their ammonifying power. The results are given in Table VI.

It is seen from this table that the different groups of organisms behaved differently when kept in culture on artificial media for a certain length of time. The Mucorales decreased their power to ammonify only in very few instances, while in several cases a stronger power to liberate ammonia is found after the organisms have been kept in culture for six months, than soon after they have been isolated from the soil. The reverse is true with the Penicillia, some of which seemed to have lost almost entirely their ammonifying power, while, with only one exception, every one of them gave, with both dried blood and cottonseed meal, smaller quantities of ammonia at the later period. The few other organisms, besides the Mucorales and the Penicillia, which have been tested, behaved like the Penicillia, e. g., decreased in their power to liberate free ammonia after being kept in culture on artificial media for six months. However, the difference in numbers of spores used for inoculation might have had something to do with the difference in results.

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C. Diastase Secretion and Cellulose Decomposition

As was seen from the brief historical review presented in the first part of the paper, the fungi seem to play an important part in the decomposition of cellulose. Out of the fungi isolated from the soil the author selected 22 species, representing the different groups of the soil fungi, to be tested out for their power to hydrolize starch and decompose cellulose. Starch and cellulose agar were made up according to the formulae given by McBeth and Scales (72). The organisms were inoculated into sterile Petri dishes containing the proper agar; the starch plates were then incubated for 6 days at 22° C., and the cellulose plates for 15 days at 30° C. The starch plates were examined after 6 days for the production of an enzymic ring around the colony. This was clearly seen even with the naked eye, but a few drops of iodine solution well defined the enzymic zone. The cellulose plates were examined under the microscope, and where cellulose decomposition had taken place the threads could be found broken down and destroyed.

TABLE VII

DIASTASE SECRETION AND CELLULOSE DECOMPOSITION BY SOIL FUNGI[1]

Name of Organism	Enzymic zone of diastase activities in 6 days	Cellulose decomposition in 15 days
Penicillium sp. (9-24)	—	++
Penicillium sp. (B. 9)	—	++
Penicillium sp. (B. 12)	—	++
Penicillium decumbens	—	+
Penicillium digitatum	6 mm.	—
Scopulariopsis brevicaule, var.	4 mm.	+
Penicillium glaber	1.5 mm.	+
Aspergillus fumigatus	—	++
Aspergillus diversicolor	—	—
Aspergillus sp. (B. 16)	2 mm.	+
Aspergillus calyptratus	1 mm.	++
Absidia sp.	—	—
Mucor plumbeus	—	—
Mucor hiemalis	—	—
Rhizopus nodosus	—	—
Melanconium sp.	—	++
Trichoderma Koningi	—	++
Verticillium sp.	6 mm	++
Basisporium gallarum	—	++
Alternaria humicola	—	++
Fusarium bullatum	—	++
Cephalosporium curtipes (?)	—	—

[1] + indicates cellulose decomposition. ++ very strong decomposition.
— no cellulose decomposition, also no diastase secretion.

Comparing the data presented in Table VII, one can see that the organisms do not have necessarily combined the power to secrete diastase and decompose cellulose. Some of the strongest cellulose decomposing organisms, such as the Melanconium, Trichoderma, Fusarium, and

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others, did not secrete any diastase under the conditions at hand, and no clearing was produced in the starch around the colony. The four Mucorales tested neither hydrolized the starch nor decomposed the cellulose.

Summary of the Physiological Studies

1. Five fungi isolated from the soil and representing distinct groups of organisms were not found to fix any appreciable quantities of atmospheric nitrogen, which would not lie within the analytical error.

2. The fungi of the soil are very strong ammonifiers, most of them liberating larger quantities of ammonia than the strong ammonifying bacteria, when tested under similar conditions.

3. The *Trichoderma Koningi* proved to be, under the conditions at hand, the strongest ammonifying organism; the Penicillia differing with the different species, most of them being comparatively weak ammonifiers; the Mucorales are fairly strong ammonifiers, the different species not differing so much from one another as the Penicillia.

4. The growing of the organisms on artificial culture media for six months affected the ammonifying power of the organisms differently: while that of the Mucorales was hardly affected, or was even beneficial, that of the Penicillia and other organisms tested, was detrimental, their power decreasing with almost all organisms.

5. Most of the fungi are very strong cellulose decomposers, 15 out of 22 organisms tested prove to decompose the cellulose rather rapidly; most of the fungi have a rather weak ability to secrete diastase, only 6 out of 22 organisms forming an enzymic ring in the starch medium.

GENERAL SUMMARY

1. The fungi of the soil represent a numerous group of organisms found in all the soils studied in numbers large enough to warrant a conclusion that they probably play an important part in the fertility of the soil.

2. There does not seem to be any distinct difference between the species of fungi found in cultivated soils and those in uncultivated soils, though each soil seems to have a more or less characteristic fungus flora: for example, the cultivated orchard soil has a great abundance of Mucorales, while the forest, uncultivated soil, has an abundance of Penicillia and Trichodermae. This might be due rather to the soil reaction, methods of manuring, and crop grown upon the soil, than to the cultivation itself.

3. The numbers of fungi decrease rapidly with depth, so that at 12 to 20 inches below the surface very few fungi can be found, the largest numbers occurring within the upper four inches of soil. As to the species, no distinct differences among the organisms were found with the different soil depths, except that in the subsoils of most of the soils studied, *Zygorhynchus Vuilleminii* was found to be present often as the only organism, when soil was inoculated directly upon sterile medium.

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4. Over one hundred distinct species of fungi were isolated from the soil, belonging to 31 genera, many of the species being isolated from several of the different soils.

5. Many pathogenic fungi, such as different Fusaria, Alternaria, Aspergilli, Coniothyrium, and others, have been isolated from the soil, a fact which leads one to think that they pass certain stages of their life history in the soil, or are able to live saprophytically in the soil, and perhaps play a part in its fertility.

6. The study of the physiological activities of the fungi pointed out the fact that they do not play a very great, if any, part in the fixation of atmospheric nitrogen, but they do prove to be able to decompose organic matter rapidly and liberate ammonia, under laboratory conditions. Many of them prove to be strong decomposers of cellulose, though fewer of them hydrolize starch.

The question, "Is there any so-called fungus flora of the soil?" cannot as yet be answered in the affirmative till more work has been done with soils collected from different parts of the world. But it is seen from the data at hand that there is a rather distinct fungus flora of the soils studied, and this holds particularly true with regard to certain organisms. The importance of the fungi in the soil seems to lie in the formation of humus and in the liberation of ammonia, which can then be utilized by the higher plants, either directly, or after it was changed by other organisms into nitrates. The numerous species of soil fungi isolated and the large numbers of them supply an impetus to a further study of these organisms, which will help to solve the problem of their importance in the soil.

Literature Cited

(1) Adametz, L.
1886. Untersuchungen über die niederen Pilze der Aekerkrume. Inaug. Diss. Leipzig, 78 p., 2 pl.

(2) Appel, O.
1906. Beiträge zur Kenntnis der Fusarien und der von ihnen hervorgerufenen Pflanzenkrankheiten. *In* Arb. K. Gsnthtsamt., Biol. Abt., Bd. 5, no. 4, p. 155-156.

(3) Appel, O.
1907. Untersuchungen über die Gattung Fusarium. *In* Mitt. Biol. Reichanst. Land u. Forstw, Bd. 4, p. 31-33.

(4) Appel, O., and Wollenweber, H. W.
1910. Grundlagen einer Monographie der Gattung Fusarium (Linn). *In* Arb. K. Gendhtsamt., Biol. Abt., Bd. 8, p. 141-156.

(5) Bainier, G.
1903. Sur quelques espèces de Mucarinées nouvelles ou peu connues. *In* Bul. Soc. Mycol. France, t 19, p. 153-172.

(6) Bainier, G.
1907. Scopulariopsis (Penicillium pro arte), genre nouveau de Mucorinées. *In* Bul. Soc. Mycol. France, t. 23, p. 98.

Digitized by Google

(7) DE BARY, A.
1863. Recherches sur le development de quelques champignons parasites. *In* Ann. Sci. Nat. Bot., s. 4, t. 20, p. 1-148.
(8) DE BARY, A.
1886. Uber einiger Sclerotinien und Sclerotinienkrankeiten. *In* Bot. Ztg., Bd. 44, no. 22, p. 377-387.
(9) BECKWITH, T. D.
1911. Root and culm infections of wheat by soil fungi in North Dakota. *In* Phytopathology, v. 1, p. 170-176.
(10) BEIJERINCK, W.
1901. Uber oligotrophile Mikroben. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 7, p. 561.
(11) BEIJERINCK, W.
1904[1]. —— *In* Arch. Néerland. Sci. Exact. et Nat., s. 2, t. 8.
(12) BERTHELOT, M.
1893. Recherches nouvelles sur les microörganismes fixateurs de l'azote. *In* Compt. Rend. Acad. Sci. [Paris], t. 116, p. 842-849.
(13) BOLLEY, H. L.
1913. Wheat: soil troubles and seed deteriorations. N. Dak. Agr. Exp. Sta. Bul. 107.
(14) BOURQUELOT, ÉMILE.
1893. Les ferments soluble de l' *"Aspergillus niger."* *In* Bul. Soc. Mycol. France, t. 9, p. 230-238.
(15) BREFELD, OSCAR.
1872. Botanische Untersuchungen über Schimmelpilze. Heft 1, Zygomyceten, p. 1-64.
(16) BROWN, P. E.
1913. Methods for bacteriological examination of soils. Iowa Agr. Exp. Sta. Research Bul. 11, p. 381-407.
(17) BUTKEWITSCH, W.
1903. Umwandlung der Eiweissstoffe durch die niederen Pilze, u. s. w., *In* Jahrb. Wiss. Bot., Bd. 38, p. 147-240.
(18) BUTLER, E. J.
1907. An account of the genus Pythium and some Chytridiaceae. *In* Mem. Dept. Agr. India, Bot. Ser. 5, v. 1, p. 1-160.
(19) CARBONE, D.
1910. Sulla decomposizione aerobica della cellulosa. *In* Bol. Soc. Med. Pavia, t. 1, p. 99-109, 375-385.
(20) CARBONE, D., and MARINCOLA-CATTANEO, R.
1908. Sull 'influenza dell 'osigeno nella decomposizione dei vegetali. *In* Arch. Farmacol. Sper. e Sci. Aff., t. 7, p. 265-301.
(21) CHIVERS, A. H.
1915. A monograph on the genera Chaetomium and Ascotricha. *In* Mem. Torrey Bot. Club, v. 14, no. 3, p. 155-240, pl. 6-17.
(22) CHRISTENSEN, H. R.
1910. Uber den Einflusz der Humusstoffe auf die Ureumspaltung. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 27, p. 336-362.
(23) COOK, M. T., and TAUBENHAUS, J. J.
1911. Trichoderma Koningi the cause of a disease of sweet potatoes. *In* Phytopathology, v. 1, no. 6, p. 184-189.

[1] Title of article not available.

Digitized by Google

(24) COOK, M. T., and TAUBENHAUS, J. J.
1911. The relation of parasitic fungi to the content of the cells of the host plant. *In* Del. Agr. Exp. Sta. Bul. 91, p. 10.
(25) COUPIN, H., and FRIEDAL, J.
1904. Sur la biologie du Sterigmatocystis versicolor. *In* Compt. Rend. Acad. Sci. [Paris], t. 138, p. 1118-1120.
(26) CZAPEK, F.
1902. Untersuchungen über die Stickstoffgewinnung und Eiweissbildung der Schimmelpilze. *In* Beitr. Chem. Physiol. u. Path., Bd. 2, p. 55-91.
(27) DALE, E.
1912. On the fungi of the soil. *In* Ann. Mycol. Bd. 10, p. 452-477, pl. 9-14.
1914. On the fungi of the soil. *In* Ann. Mycol., Bd. 12, p. 33-62, pl. 1-5.
(28) DASZEWSKA, W.
1913. Étude sur la désagrégation de la cellulose dans la terre de bruyère et la toube. *In* Univ. Genève. Inst. Bot. s. 8, fas. 8, p. 238-316.
(29) DOX, A. W., and NEIDIG, R. E.
1911. Pentozans in lower fungi. *In* Jour. Biol. Chem., v. 9, p. 267-269.
(30) DUGGAR, B. M., and KNUDSON, L.
1911. Relation of certain fungi to nitrogen fixation. Abs. *in* Science, n. s. v. 32, p. 191.
(31) EHRENBERG, P.
1907. Die Bewegung des Ammoniakstickstoffs in der Natur. *In* Mitt. Landw. Inst. Breslau, Bd. 4, p. 47-300.
(32) EHRLICH, F., and JACOBSEN, K. A.
1911. Uber die Umwandlung von Aminosauren in Oxysäuren durch Schimmelpilze. *In* Ber. Deut. Chem. Gesell., Bd. 44, p. 888-897.
(33) ENGLER, A., and PRANTL, K.
1897. Die natürlichen Pflanzenfamilien. Leipzig.
(34) FISCHER, A.
1892. Phycomycetes. *n* Rab. Krypt. Flora, Abt. 4, p. 178-213.
(35) FISCHER, H.
1909. Bakteriologisch-chemische Untersuchungen: Bakteriologisches Teil. *In* Landw. Jahrb., Bd. 38, p. 355-368.
(36) FRANK, B.
1885. Uber die auf Wurzelsymbiose beruhende Ernährung gewissen Baume durch unterirdische Pilze. *In* Ber. Deut. Bot. Gesell., Bd. 3, p. 128.
(37) FRANK, B.
1888. Uber die physiologische Bedentung der Mycorhiza. *In* Ber. Deut. Bot. Gesell., Bd. 6, p. 248-269.
(38) FRANK, B.
1893. Die Assimilation des freien Stickstoffs durch die Pflanzenwelt. *In* Bot. Ztg., Bd. 51, p. 139-156.
(39) FREMY, E.
1848. Memoire sur la maturation des fruits. *In* Ann. Chim. et Phys., s. 3, t. 24, p. 5-58.
(40) FROELICH, H.
1908. Stickstoffbindung durch einige auf abstorbenen Pflanzen häufige Hyphomyceten. *In* Jahrb. Wiss. Bot., Bd. 45, p. 256-302.
(41) GODDARD, H. M.
1913. Can fungi living in agricultural soil assimilate free nitrogen? *In* Bot. Gaz., v. 56, no. 4, p. 249-305.

Digitized by Google

(42) HAGEM, O.
1907. Untersuchungen über Norwegische Mucorineen, I. *In* Vidensk. Selsk., I Math. Naturw. Klasse, Bd. 7, p. 1-50.

(43) HAGEM, O.
1910. Untersuchungen über Norwegische Mucorineen, II. *In* Vidensk. Selsk., I Math. Naturw. Klasse, Bd. 10, No. 4, p. 1-152.

(44) HAGEM, O.
1910. Neue Untersuchungen über Norwegische Mucorineen. *In* Ann. Mycol., Bd. 8, p. 265-286.

(45) HALL, A. D., MILLER, N. H., and GIMINGHAM, C. T.
1908. Nitrification in acid soil. *In* Proc. Roy. Soc., B., v. 80, p. 196-211.

(46) HANZAWA, J.
1915. Studien über einige Rhizopus Arten. *In* Mycol. Centbl., Bd. 5, Heft 5, p. 230.

(47) HEINZE, B. H.
1906. Sind Pilze imstande den elementaren Stickstoff der Luft zu verarbeiten und den Boden an Gesamstickstoff anzureichen. *In* Ann. Mycol., Bd. 4, p. 41-63.

(48) HEINZE, B. H.
1906. Einige Beiträge zur mikrobiologischen Bodenkunde. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 16, p. 703-711.

(49) HEINZE, B. H.
1906. Uber die Stickstoffassimilation durch neidere Organismen. *In* Landw. Jahrb., Bd. 35, p. 889-910.

(50) HILTNER, L.
1903. Uber die biologische und physiologische Bedeutung der endotropen Mycorhizen. *In* Naturw. Ztschr. Land. u. Forstw., Bd. 1, p. 9.

(51) HÖVELER, W.
1892. Uber die Verwerthung des Humus bei der Ernährung der chlorophyll führenden Pflanzen. *In* Jahrb. Wiss. Bot., Bd. 24, p. 283-316.

(52) VAN ITERSON, C. J.
1904. Die Zersetzung von cellulose durch aërobe Microörganismen. *In* Centbl. Bakt. [etc], Abt. 2, Bd. 11, p. 689-698.

(53) JENSEN, C. N.
1912. Fungus flora of the soil, N. Y. (Cornell) Agr. Exp. Sta. Bul. 315, p. 415-501, fig. 101-134.

(54) JODIN, H.
1862. Du rôle physiologique de l'azote. *In* Compt. Rend. Acad. Sci. [Paris], t. 55, p. 612.

(55) KAPPEN, H.
1910. Uber die Zersetzung des Cyannmids durch Pilze. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 26, p. 633-643.

(56) KELLERMAN, K. F.
1912. Formation of Cytase by Penicillium pinophilum. U. S. Dept. Agr. Bur. Plant Indus. Circ. 113, p. 29-31.

(57) KOCH, A., and KOSSOWITSCH, P.
1893. Uber die assimilation von freiem Stickstoff durch Algen. *In* Bot. Ztg., Bd. 51, Abt. 2, p. 321-325.

(58) KOHSHI, OHTA.
1911. Uber die fettzehrenden Wirkungen der Schimmelze nebst dem Verhalten des Organfettes gegen Fäulnis. *In* Biochem. Ztsch., Bd. 31, p. 177-194.

Digitized by Google

(59) KONING, C. J.
1904[1]. ——*In* Arch. Néerland. Sci. Exact. et Nat., s. 2, t. 9, p. —
(60) KOPELOFF, N.
1916. The effect of soil reaction on ammonification by certain soil fungi. *In* Soil Sci., v. 1, p. 541-574.
(61) KOSSOWICZ, A.
1914. Zur Frage der Assimilation des elementaren Stickstoffs durch Hefen und Schimmelpilze. *In* Biochem. Ztschr., Bd. 64, p. 82-85.
(62) KOSSOWITSCH, P.
1894. Untersuchungen über die Frage, ob die Algen freien Stickstoff fixiren. *In* Bot. Ztg., Bd. 52, p. 97-116.
(63) KOSTYSTCHEFF, P. A.
1891. Recherches sur la formation et les qualités de l'humus. *In* Ann. Agron., t. 17, p. 17-38.
(64) LATHAM, M. E.
1909. Nitrogen assimilation of Sterigmatocystis niger and the effect of chemical stimulation. *In* Bul. Torrey Bot. Club, v. 36, p. 235-244.
(65) LAURENT, E.
1890. La reduction des nitrates en nitrites par les graines et les tubercules. *In* Bul. Acad. Roy. Sci. Belg., s. 3, t. 20, p. 478-485.
(66) LENDNER, A.
1908. Les Mucorinées de la Suisse. 180 p.
(67) LENDNER, A.
1910. Nouvelles contributions á la flore cryptogamique Suisse. *In* Bul. Soc. Bot. Genève, s. 2, t. 2, p. 78-81.
(68) LINDAU, G.
1907-1910. Fungi imperfecti: Hyphomycetes. Rab. Krypt. Flora, Abt. 8 & 9.
(69) LIPMAN, C. B.
1911. Nitrogen fixation by yeasts and other fungi. *In* Jour. Biol. Chem., v. 10, p. 169-182.
(70) LIPMAN, J. G., and BROWN, P. E.
1911. A laboratory guide in soil bacteriology, p. 71.
(71) MARCHAL, EMILE.
1893. Sur la production de l'ammoniaque dans le sol par les microbes. *In* Bul. Acad. Roy. Sci. Belg., t. 25, p. 727-771.
(72) MCBETH, I. G., and SCALES, F. M.
1913. The destruction of cellulose by bacteria and filamentous fungi. U. S. Dept. Agr. Bur. Plant Indus. Bul. 266.
(73) MCLEAN, H. C., and WILSON, G. W.
1914. Ammonification studies with soil fungi. N. J. Agr. Exp. Sta. Bul. 270, 39 p.
(74) MIQUEL, P.
1878. De la présence dans l'air du ferment de l'urée. *In* Bul. Soc. Chim. Paris, n.s., t. 29, p. 387-397.
(75) MOLLIARD, —.
1902. Basisporium gallarum. *In* Bul. Soc. Mycol. France, t. 18, p. 167.
(76) MÜNTZ, A., and COUDON, H.
1893. La fermentation ammoniaque de la terre. *In* Compt. Rend. Acad. Sci. [Paris], t. 116, p. 395-398.

[1] Title of article and page not available.

Digitized by Google

(77) NAMYSLOWSKI, B.
1910. Studien über Mucorineen. *In* Bul. Acad. Sci. Cracovie., Class Math. et Nat., ser. B., p. 477-520.

(78) NAMYSLOWSKI, B.
1910. Zygorhynchus Vuilleminii une nouvelle Mucorinée isolée due sol et cultivée. *In* Ann. Mycol., Bd. 8, p. 152-155.

(79) NIKITINSKY, J.
1902. Uber die Zersetzung der Huminsäure durch physikalisch-chemische Agentien und durch Microörganismen. *In* Jahrb. Wiss. Bot., Bd. 37, p. 365-420.

(80) OUDEMANS, A. C., and KONING, C. J.
1902. Prodrome d'une flore mycologique, obtenue par la culture sur gelatin préparée de la pres de Russum. *In* Arch. Néerland. Sci. Exact. et Nat., t. 7, s. 2, p. 266-298.

(81) PEROTTI, B.
1907. Uber das physiologische Verhalten des Dicyanamides mit Rücksicht auf seinen Wert als Düngemittel. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 18, p. 50-56.

(82) PURIEWITSCH, K.
1895. Uber die Stickstoffassimilation bei den Schimmelpilzen. *In* Ber. Deut. Bot. Gesell., Bd. 13, p. 342.

(83) RAMANN, E.
1905. *In* Bodenkunde. Berlin, p. 116-121.

(84) RAMANN, E., REMELÉ, C., SCHELLHORN, — and KRAUSE, M.
1900. Anzahl und Bedeutung der niederen Organismen in Wald- und Moorböden. *In* Ztschr. Forst. u. Jagdw., Bd. 31, Heft. 10, p. 575-606.

(85) REINITZER, F.
1900. Uber die Eignung der Huminsubstanzen zur Ernährung von Pilzen. *In* Bot. Ztg., Bd. 58, p. 59-73.

(86) REMY, TH.
1902. Bodenbakteriologische Studien. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 8, p. 728-735.

(87) REMY, TH.
1903. N.-Bindung dursch Leguminosen. *In* Verhandl. Gesell. Deut. Naturf. u. Arzte. Karlsbad. Teil I.

(88) RIDGWAY, R.
1912. Color standards and color nomenclature. Washington, D. C. 53 pl., 1115 colors.

(89) RITTER, G.
1909. Ammoniak und Nitrate als Stickstoffquelle für Schimmelpilze. *In* Ber. Deut. Bot. Gesell., Bd. 27, p. 582-588.

(90) ROUSSY, A.
1909. Sur la vie des champignons en milieux gras. *In* Compt. Rend. Acad. Sci. [Paris], t. 149, p. 482-484.

(91) SAIDA, K.
1901. Uber die Assimilation freien Stickstoffs durch Schimmelpilze. *In* Ber. Deut. Bot. Gersell., Bd. 19, p. 107.

(92) SCALES, F. M.
1914. The enzymes of Aspergillus terricola. *In* Jour. Biol. Chem., v. 19, no. 4, p. 459-472.

Digitized by Google

(93) SCALES, F. M.
1915. Some filamentous fungi tested for cellulose destroying power. *In* Bot. Gaz., v. 60, no. 2, p. 149-153.
(94) SCHELLENBERG, H. C.
1908. Untersuchungen über das Verhalten einiger Pilze gegen Hemizellulosen. *In* Flora, Bd. 98, no. 3, p. 257-308.
(95) SCHERPE, R.
1909. Uber den Einflus des Schwefelkohlenstoffs auf die Stickstoffumsetzungsvorgänge im Boden. *In* Arb. K. Gsndhtsamt., Biol. Abt., Bd. 7, p. 353-428.
(96) SCHLÖSING, TH., and LAURENT, E.
1892. Récherches sur la fixation de l'azote libre par les plantes. *In* Compt. Rend. Acad. Sci. [Paris], t. 115, p. 659-732.
(97) SEMEL, O.
1898[1]. —— *In* Ann. Pharm., t. 4, p. 279.
(98) SHERBAKOFF, C. D.
1915. Fusaria of potatoes. N. Y. (Cornell) Agr. Exp. Sta. Mem. 6, p. 85-270.
(99) SHIBATA, K.
1904. Uber das Vorkommen vom Amide spaltenden enzymen bei Pilzen. *In* Beitr. Chem. Physiol. u. Path., Bd. 5, p. 384-394.
(100) SOPP, O. IOHAN OLSEN.
1912. Monographie der Pilzgruppe Penicillium, etc. *In* Vidensk. Selsk. Skr. I Math. Naturw. Klasse, no. 11.
(101) STAHEL, G.
1911. Stickstoffbindung durch Pilze. *In* Jahrb. Wiss. Bot., Bd. 49, p. 579-615.
(102) STOKLASA, J.
1900. Assimilieren die Alinitbakterien den Luftstickstoff? *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 6, p. 22-24.
(103) TERNETZ, C.
1904. Die Assimilation des atmospherischen Stickstoffs durch einen torfbemohnenden Pilz. *In* Ber. Deut. Bot. Gesell., Bd. 22, p. 267-274.
(104) THOM, CH.
1910. Cultural studies of species of Penicillium. U. S. Dept. Agr. Bur. Anim. Indus. Bul. 118, 107 p.
(105) THOM, CH.
1915. The Penicillium Luteum-Purpurogenum group. *In* Mycologia, v. 7, no. 3, p. 134-142.
(106) VAN TIEGHEM, M. PH.
1875. Nouvelles recherches sur les Mucorinées. *In* Ann. Sci. Nat. Bot., s. 6, t. 1, p. 1-175.
(107) VAN TIEGHEM, M. PH.
1876. Troisième memoire sur les Mucorinées. *In* Ann. Sci. Nat. Bot., s. 6, t. 4, p. 312-398.
(108) TRAAEN, A. E.
1914. Untersuchungen über Bodenpilze aus Norwegen. *In* Nyt. Magaz. Naturw. Christiania, Bd. 52, pt. 1, p. 20-121.
(109) VUILLEMIN, P.
1887. Etudes biologiques sur les champignons. *In* Bul. Soc. Sci. Nancy, t. 19, p. 1-129.

[1] Title of article not available.

Digitized by Google

(110) VUILLEMIN, P.
1903. Importance taxonomique de l'appareil zygosporé des Mucorinées. *In* Bul. Soc. Mycol. France, t. 19, p. —.
(111) VUILLEMIN, P.
1904. Sur les variations spontanées du Sterigmatocystis versicolor. *In* Compt. Rend. Acad. Sci. [Paris], t. 138, p. 1350-1352.
(112) WAKSMAN, S. A.
1915. Bacteria, actinomyces, and fungi in the soil. Reported before the 17th annual meeting of the American Bacteriological Society at Urbana, Ill.
(113) WAKSMAN, S. A.
1916. Bacterial numbers in soils, at different depths, and in different seasons of the year. *In* Soil Sci., v. 1, p. 363-380.
(114) WAKSMAN, S. A.
1916. Do fungi actually live in the soil and produce mycelium? *In* Science (soon to appear).
(115) WAKSMAN, S. A., and COOK, R. C.
1916. Incubation studies with Soil Fungi. *In* Soil Science, v. 1. p. 275-284.
(116) WARD, H. M.
1888-9. A lily disease. *In* Ann. Bot., v. 2, p. 319-382.
(117) WARD, H. M.
1898. Penicillium as a wood destroying fungus. *In* Ann. Bot., v. 12, p. 565-566.
(118) WEHMER, C.
1895. Zur Kenntnis der einheimischer Pilze, Heft 2, 144 p., tab. 3, fig. 16-19.
(119) WEHMER, C.
1903. Der Mucor der Hanfrötte, M. heimalis, nov. spec. *In* Ann. Mycol., Bd. 1, p. 37-41.
(120) WEHMER, C.
1905-1907. Morphologie und Systematik der Familie der Aspergillaceen. *In* Lafar's Hand. Tech. Mykol., Bd. 4, p. 192-238.
(121) WEHMER, C.
1914. Bemerkungen zur Systematik der Gattung Penicillium. *In* Ber. Deut. Bot. Gesell., Bd. 32, Heft 5, p. 373-384.
(122) WENT, F. A. F. C.
1901. Uber den Einflusz der Nahrung und die Enzymbildung durch Monilia sitophila (Mont) Sacc. *In* Jahrb. Wiss. Bot., Bd. 36, p. 611-664.
(123) WESTLING, R.
1911. Uber die grünen Species der Gattung Penicillium. Inaug. Dissert. Upsala. *In* Ark. Bot., Bd. 11, p. 1-156.
(124) WILFERTH, H., and WIMMER, G.
1907. Uber den Einfluss der Mineraldüngung auf die Stickstoffbindung durch niedere Organismen im Boden. *In* Landw. Vers. Sta., Bd. 67, p. 27-50.
(125) WINOGRADSKY, S. N.
Recherches sur l'assimilation de l'azote libre de l'atmosphère par les microbes. *In* Arch. Sci. Biol., t. 4. pl. 3.

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PLATE I

Fig. 1.—*Absidia Orchidis.* Different types of branching of the sporangiophore (x 104).
Fig. 2.—*Absidia Orchidis.* Types of columellae (x 600).
Fig. 3.—*Absidia Orchidis.* Spores (x 1200).
Fig. 4.—*Mucor botryoides.* Branching of sporangiophore, carrying columellae (x 600).
Fig. 5.—*Mucor botryoides.* Spores (x 1200).
Fig. 6 a-b.—*Mucor saturninus.* Sporangiophores (x 200).
Fig. 7.—*Mucor saturninus.* Columella (x 300).
Fig. 8.—*Mucor saturninus.* Spore (x 1200).
Fig. 9 a-b.—*Mucor* sp. (C. 44). Sporangiophores straight and curved type (x 104).
Fig. 10.—*Mucor* sp. (C. 44). Columellae of the straight and curved type of sporangiophores (x 1200).
Fig. 11.—*Mucor* sp. (C. 44). Spores (x 1200).
Fig. 12.—*Mucor* sp. (D. 28). Columellae (x 600).
Fig. 13.—*Mucor* sp. (D. 28). Spores (x 1200).
Fig. 14.—*Mucor* sp. (D. 28). Portion of mycelium, showing oil globules and chlamydospores (x 600).

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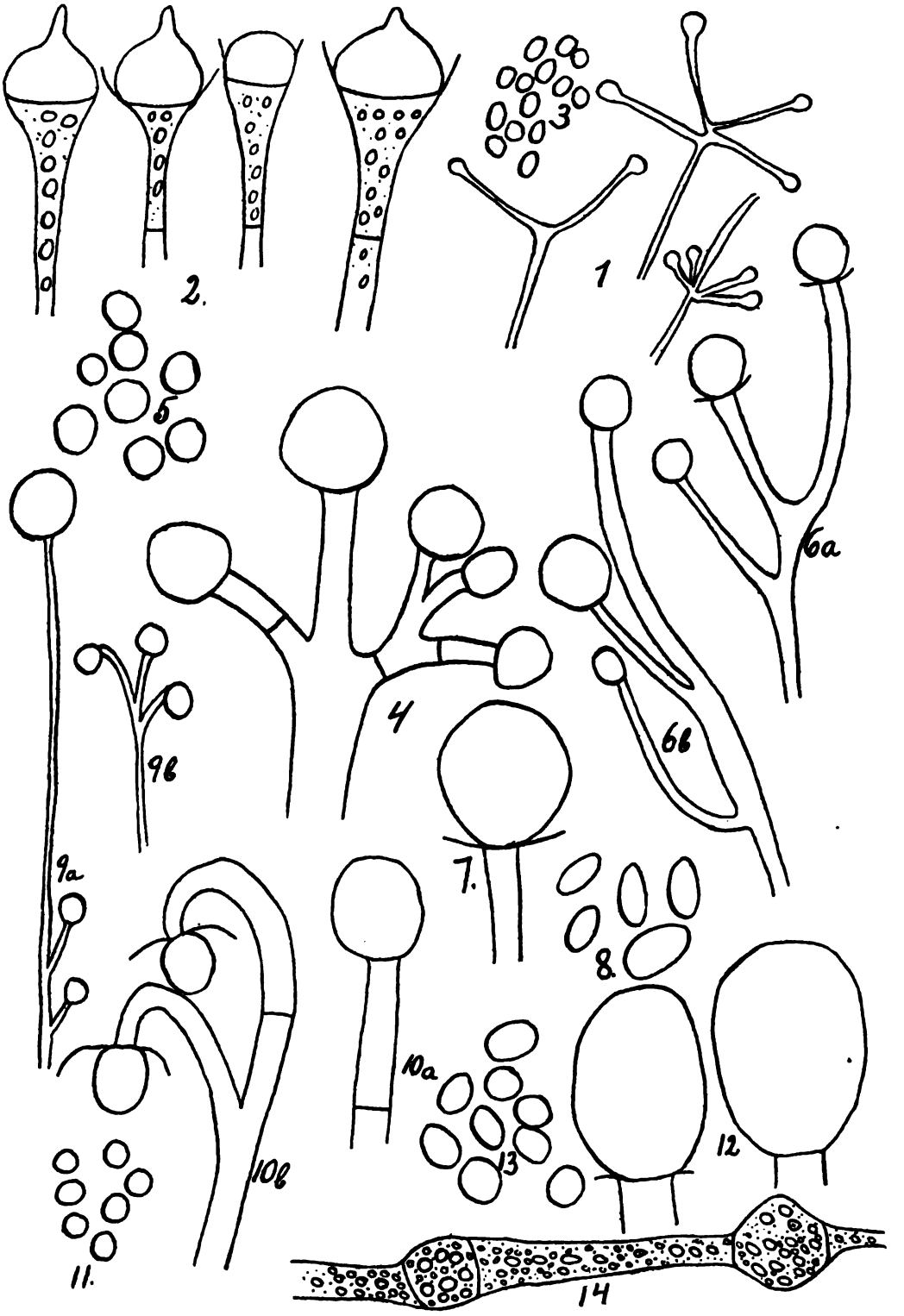

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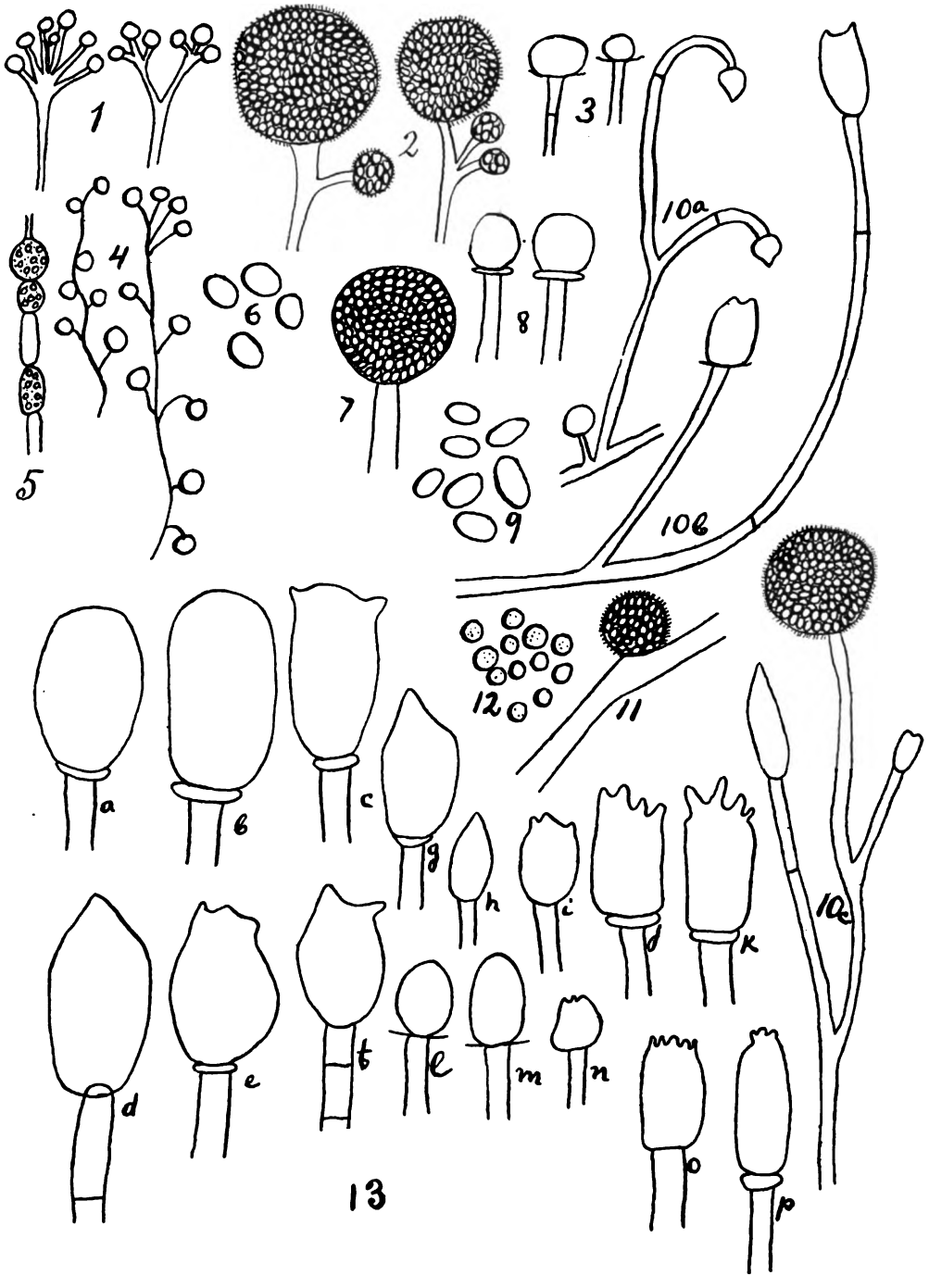

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PLATE II

Fig. 1.—*Mucor Glomerula.* Branching of Sporangiophores (x 60).
Fig. 2.—*Mucor Glomerula.* Sporangia (x 310).
Fig. 3.—*Mucor Glomerula.* Columellae (x 310).
Fig. 4.—*Mucor circinelloides.* Sporangiophores branched sympodially (x 60).
Fig. 5.—*Mucor circinelloides.* Portion of mycelium, showing chlamydosphores (x 310).
Fig. 6.—*Mucor circinelloides.* Spores (x 1200).
Fig. 7.—*Mucor hiemalis.* Sporangium (x 310).
Fig. 8.—*Mucor hiemalis.* Columellae (x 310).
Fig. 9.—*Mucor hiemalis.* Spores (x 1200).
Fig. 10.—*Mucor plumbeus,* showing branching of sporangiophores, 10 c, showing one sporangium and two columellae (x 310).
Fig. 11.—*Mucor plumbeus.* Portions of sporangiophore bearing a sessile sporangium (x 310).
Fig. 12.—*Mucor plumbeus.* Spores (x 600).
Fig. 13 a-p.—*Mucor plumbeus.* Types of columellae (x 600). a, o, p from Strain I; b, c, g, h from Strain IV; e, f, d from Strain (C. 20); l, m, n from Strain (D. 23); j, k from Strain (C. 22).

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PLATE III

Fig. 1 a, b, c.—*Zygorhynchus Vuilleminii.* Formation of zygospores (x 310).

Fig. 2.—*Zygorhynchus Vuilleminii.* Portion of sporangiophore, showing ripe sporangium and zygospore (x 600).

Fig. 3.—*Zygorhynchus Vuilleminii.* Branching of sporangiophore (x 600).

Fig. 4.—*Zygorhynchus Vuilleminii.* Portion of mycelium showing chlamydospores (x 310).

Fig. 5.—*Zygorhynchus Vuilleminii.* Spores (x 1200).

Fig. 6.—*Monilia humicola.* Portions of conidiiferous branches showing formation of conidia (x 600).

Fig. 7.—*Aspergillus* sp. (C. 19). Vegetative hyphae and conidiophores (x 310).

Fig. 8.—*Aspergillus* sp. (C. 19). Greatly enlarged swelling of conidiophore showing sterigmata and conidia (x 1200).

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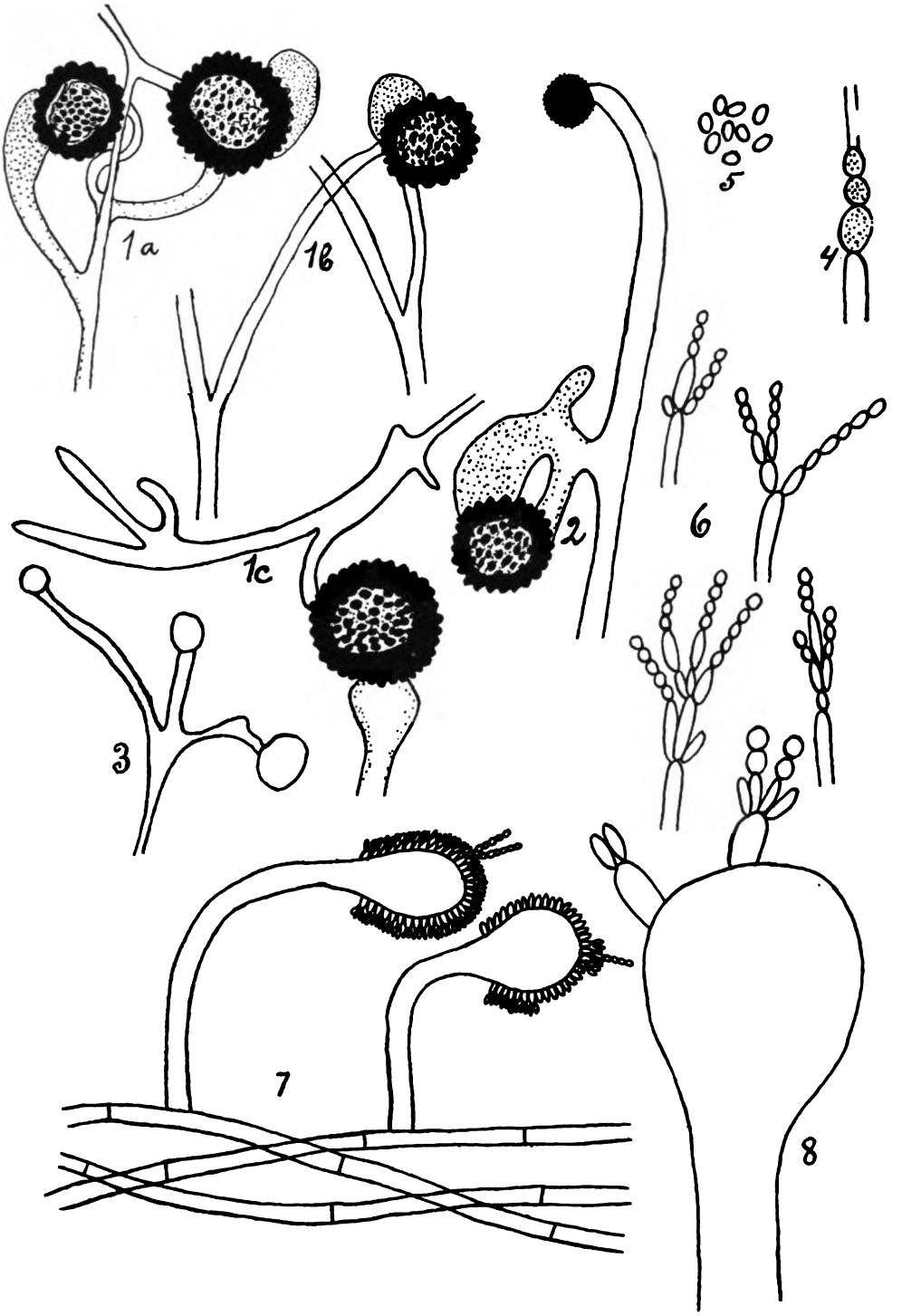

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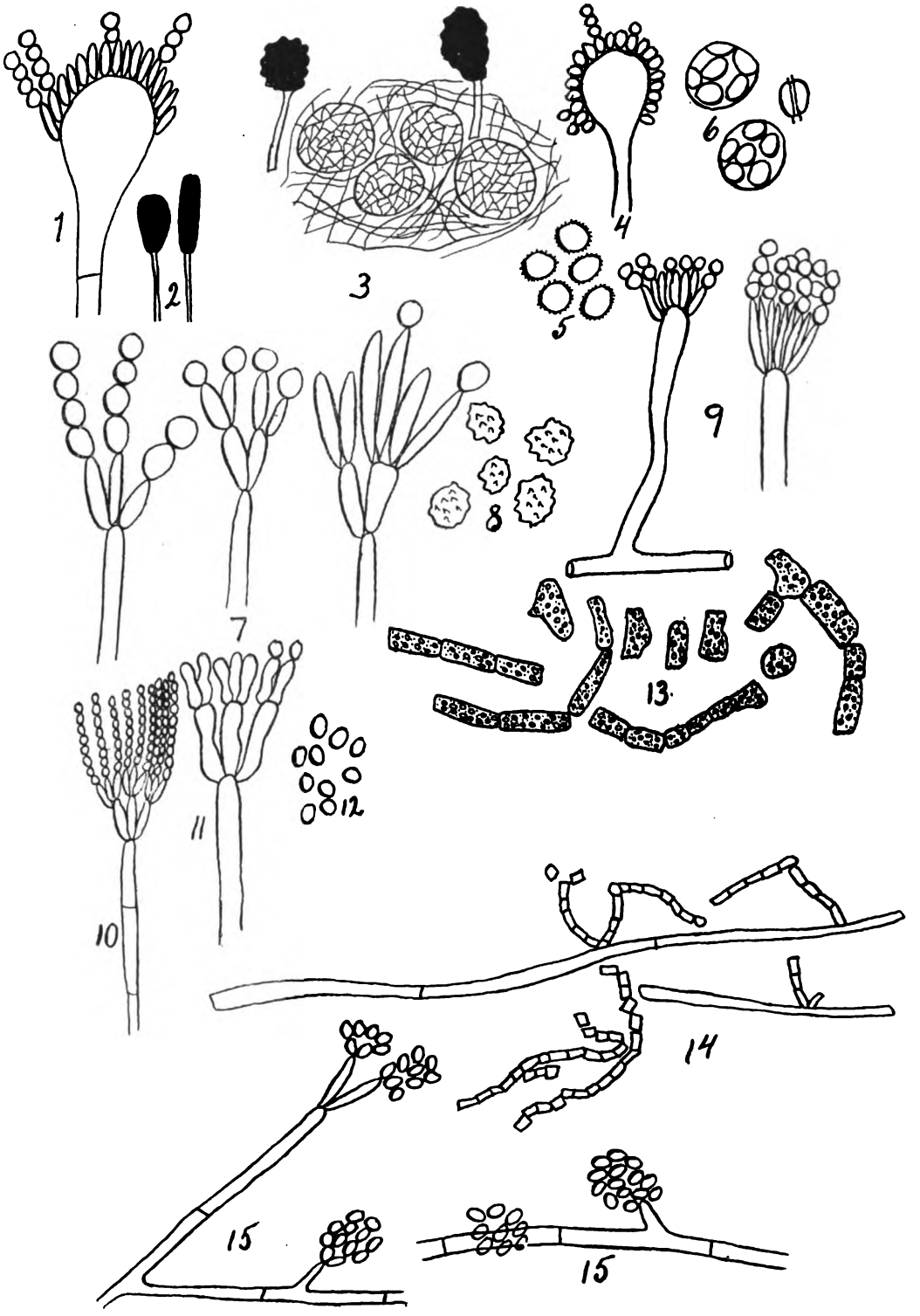

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PLATE IV

Fig. 1.—*Aspergillus calyptratus.* Portion of conidiophore showing swelling, sterigmata, and conidia (x 1200).

Fig. 2.—*Aspergillus calyptratus.* Conidial fructifications (x 104).

Fig. 3.—*Aspergillus repens* (from raisin agar). A portion of the mycelial surface with perithecia and canidiophores (x 104).

Fig. 4.—*Aspergillus repens.* Conidial swelling with sterigmata (x 600).

Fig. 5.—*Aspergillus repens.* Conidia (x 1200).

Fig. 6.—*Aspergillus repens.* Asci containing the ascospores also free ascospore (x 1200).

Fig. 7.—*Scopulariopsis brevicaule* (n. var. ?) Conidiophores with conidial chains of smooth spores (x 600).

Fig. 8.—*Scopulariopsis brevicaule* (n. var. ?) Echinulate spores (x 600).

Fig. 9.—*Penicillium glaber.* Portions of conidiophores showing sterigmata and spores (x 600).

Fig. 10.—*Penicillium desiscens.* Portion of conidiophore magnified (x 600).

Fig. 11.—*Penicillium desiscens.* Highly magnified portion of conidiophore to show sterigmata (x 1200).

Fig. 12.—*Penicillium desiscens.* Spores (x 1200).

Fig. 13.—*Oidium* sp. (A. 30). The breaking of the hyphae into oidia (x 570).

Fig. 14.—*Oidium lactis.* Hyphae and abundant formation of side branches, resulting in the formation of odia (x 310).

Fig. 15.—*Cephalosporium* sp. (D. 32). Formation of conidial heads at the tip of the branches or on the main hyphae (x 600).

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PLATE V

Fig. 1.—*Cephalosporium* sp. (G. 23). Branching of conidiophore and formation of heads of conidia at the tip of the branches (x 310).

Fig. 2.—*Cephalosporium* sp. (G. 23). Spores (x 1200).

Fig. 3.—*Cephalosporium* (n. sp. ?) (C. 56). Branching of sporangiophore and the grouping of spores into heads (x 600).

Fig. 4.—*Cephalosporium* (n. sp. ?) (C. 56). Branching of conidiophore, showing heads of spores surrounded by slime in heads (x 600).

Fig. 5.—*Cephalosporium* (n. sp. ?) (C. 56). Spores (x 1200).

Fig. 6.—*Zygodesmus* sp. Portion of mycelium with side branches carrying spores (x 104).

Fig. 7.—*Zygodesmus* sp. Tips of the branches of the conidiophore, surrounded by spores (x 600).

Fig. 8.—*Zygodesmus* sp. Three spores of the major strain Oc (x 1200).

Fig. 9.—*Zygodesmus* sp. Four spores of the minor strain B (x 1200).

Fig. 10.—*Coniothyrium Fuckelii* (?). Pycnidium surrounded by mycelium (x 60).

Fig. 11.—*Coniothyrium Fuckelii* (?) Portion of mycelium (x 310).

Fig. 12.—*Coniothyrium Fuckelii* (?) Spores (x 600).

Fig. 13.—*Cladosporium epiphyllum*. Portion of fruiting hyyhae and fructifications.

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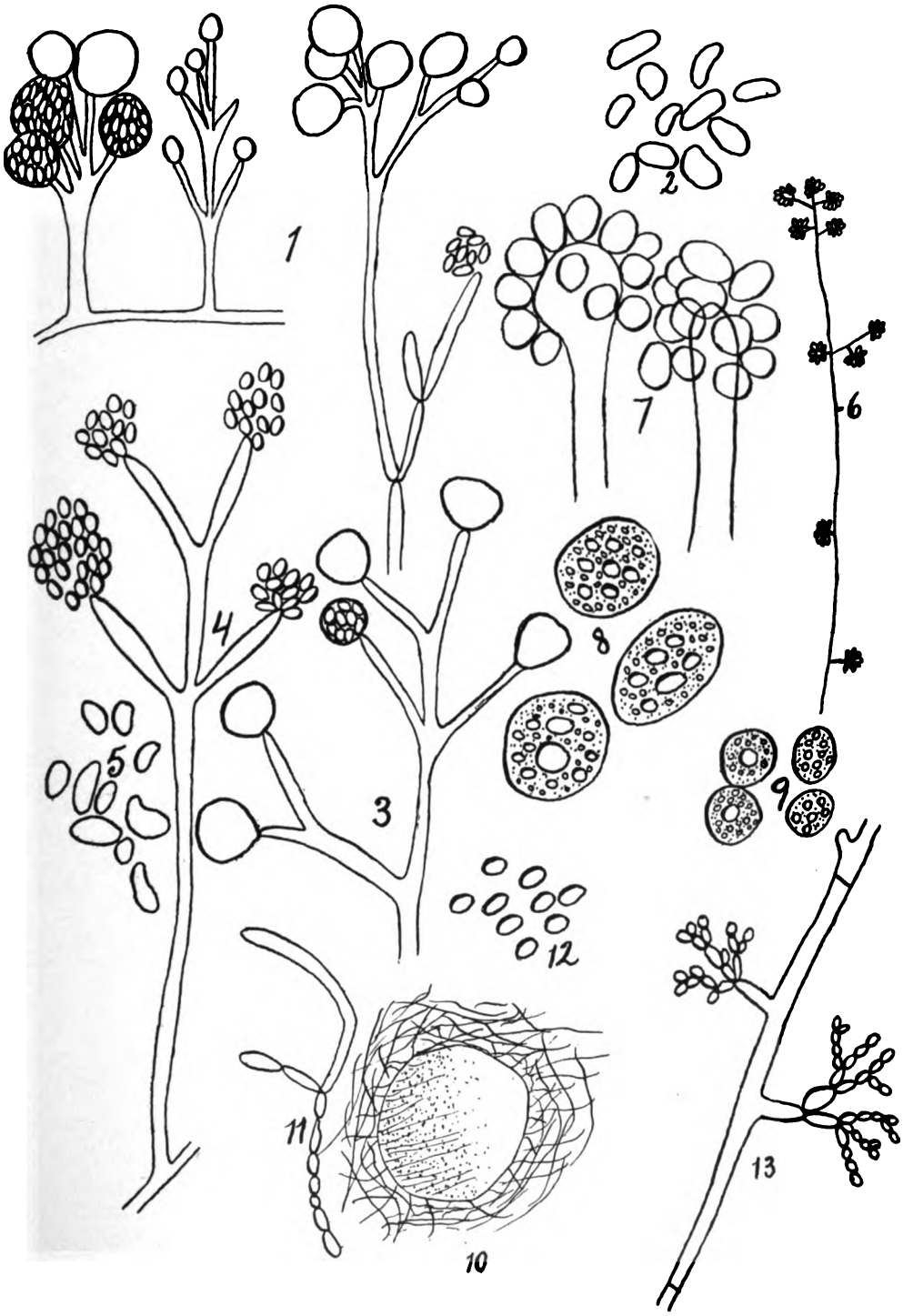

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SOURCES OF ERROR IN SOIL BACTERIOLOGICAL ANALYSIS[1]

By H. Clay Lint and David A. Coleman, *Research Fellows, Rutgers College*

Investigations in the field of soil biology have concerned themselves mostly with the measurement of the activity of microörganisms by means of chemical methods. To ascertain to what degree the various bacteriological phenomena of ammonification, azofication and azotofication,[2] etc., have taken place, recourse has been taken to the usual qualitative and quantitative methods of the analytical chemist.

One of the first surprises in store for the soil biologist who, as a rule, has had more or less training in analytical chemistry, is to the effect that good duplicate determinations, in the chemical sense, are the exception rather than the rule.

No action which depends upon vital processes for its performance can be repeated with the certainty of chemical reaction, since the slightest differences in environmental factors cause variation in the response of the micro-flora and fauna involved. Small irregularities are magnified rather than reduced when the bacteriological activity is eventually measured by chemical methods. Greater or less differences between duplicates may result from wholly obscured causes, so that the problem of obtaining closely agreeing determinations in biological work resolves itself, in the main, into one of eliminating errors in methods of technique.

Probably all investigators have taken care to use soil containers of the same type throughout one comparative investigation. In order to determine to what extent differences in the size and shape of the soil container would make manifest differences in the results, the following experiment was carried out.

Ammonification tests were made with 100-gm. portions of Norfolk sandy loam soil to which had been added 5 gm. of dried blood. The soil portions were made up to optimum moisture content, 13 per cent, with the addition of 3 c.c. of water for each gram of organic matter. Dupli-

[1] Received for publication April 15, 1916.

[2] Lipman, J. G. Suggestions concerning the terminology of soil bacteria. *In* Bot. Gaz., v. 51, p. 454-460, 1911.

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cate charges of the soil thus prepared were placed in seven different types of containers, incubated for 7 days at 22° C, and the ammonia accumulated was determined by the magnesium oxide distillation method.

The influence of the size and shape of the vessel on ammonification, all other factors being alike, is shown in Table I.

TABLE I

AMMONIFICATION IN CONTAINERS OF DIFFERENT TYPES

Lab. No.	Type of Container	Ammonia accumulated		
		Mg. N.	Mg. N.	Average
1-2	200 c.c. Erlenmeyer.....	67.82	68.04	67.93
3-4	250 c.c. Beaker	53.54	54.61	54.07
5-6	300 c.c. Erlenmeyer.....	74.02	87.70	80.86
7-8	500 c.c. Erlenmeyer.....	116.30	119.14	117.72
9-10	250 c.c. Florence.....	76.70	76.70	76.80
11-12	250 c.c. Salt mouth bottle.....	63.76	63.03	63.38
13-14	275 c.c. Tumbler.....	84.87	71.70	78.29

Apparently there exists an intimate correlation between the aeration of the soil and its ammonifying power. A difference of over 100 per cent exists between the ammonia accumulated in a 250 c.c. beaker and a 500 c.c. Erlenmeyer flask. Even between beakers, bottles, tumblers, all of approximately 250 c.c. capacity, there exists differences in some cases as great as 46 per cent. Of considerable interest is the fact that the soil portion in the 250-c.c. bottles had an ammonifying power greater than the same soil in a 250-c.c. beaker. The soil depth in the former was approximately two and one-half times as great as that in the beakers, nevertheless, more ammonia accumulated at the end of the incubation period. As can be seen from the table there is a gradual increase in the ammonifying power of the soil as the size of the Erlenmeyer flasks was increased from 200 to 500 c.c. The soil in the tumbler has a higher ammonifying power than that in any of the other containers, with the exception of the 500-c.c. Erlenmeyer flask.

Considering the ease with which the soil can be removed from this utensil, and the generally higher results obtained, together with its ability to withstand several usages, it would seem that this should be adopted as a vehicle for all biological work other than that of a pure culture nature.

The necessity, then of using containers of the same size and shape in all comparative studies becomes at once apparent. Likewise in comparing the results of various workers and the relative percentages of ammonia accumulated, it is not impossible that erroneous deductions may be made, due to no more important a factor than the size and shape of the containing vessel.

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Method of Mixing Soil and Organic Matter

Most laboratory guides recommend stirring with the soil, by means of a spatula, all materials designated to bring out specific biological activities, in order to effect a uniform mixture of the same. Where the fresh soil method[1] is used for the determination of biological activities one encounters considerable difficulty in obtaining an even incorporation of the materials to be used in the carrying out of the experiment. Wide deviations between duplicate determinations are not uncommon in such work, and to overcome these difficulties the writers have made use of the metal shakers shown in figure 1.

This shaker is commonly used in the mixing of beverages for which purpose it is extensively advertised. The metal prongs fixed to the cover serve to stir up the larger soil aggregates, thus effecting a very intimate and uniform incorporation of the specific materials with the soil. Very satisfactory results have been obtained by the use of a tumbler and the cup of the shaker with a pinch-cock inserted to churn the soil mass (fig. 1).

In order to demonstrate the advantages of the soil shaker in obtaining close duplicate determinations, the following comparison of the shaker and spatula methods of incorporating organic matter into the soil has been made.

Two soils, a Norfolk sandy loam and a Penn clay loam were used. One-hundred-gram portions of soil were mixed with 3 gm. of dried blood by means of the two devices. Each soil was used both in its air-dry state and at its optimum moisture content. Two periods of mixing for 15 seconds and 30 seconds were used with both soils in the dry and in the moist conditions. Five parallels were run for each period of mixing in both the wet and dry soils. The data for each parallel, as well as the experimental error of the average of the five, are shown in Table II. While it is admitted that the number of instances is hardly large enough in each case to render the use of any statistical method legitimate, nevertheless, it serves as a good index to the authenticity of the average and serves to emphasize the point under discussion. These comparisons were made according to the formula that the probable error (p.e.) of the average $= \pm .6745 \sqrt{\frac{\Sigma d^2}{n(n-1)}}$

[1] Loc. cit. p. 1.

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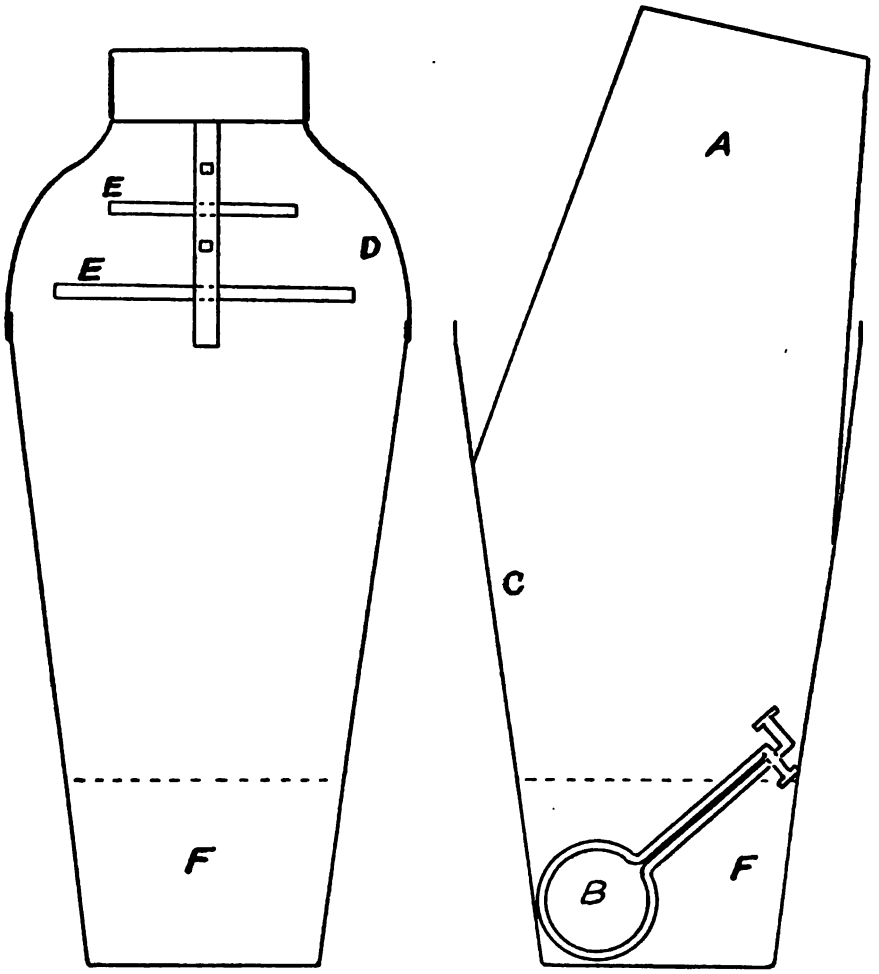

Fig. 1. Diagram of apparatus used for mixing soil.
A—Tumbler; B—Pinch-cock; C—Cup of the Shaker; D—Cap of the Shaker; E—Metal prongs for stirring the soil; F—Soil.

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TABLE II

AMMONIFICATION TESTS OF DIFFERENT METHODS OF MIXING SOILS

Condition of soil at time of Mixing	Means of Mixing	Time of Mixing	Mg. N. accumulated as NH_3 in 7 days					
			Norfolk Sandy Loam			Penn Clay Loam		
			Det.	Average	Probable error of Av. + or —	Det.	Average	Probable error of Av. + or —
Air Dry	Shaker	30 sec.....	88.30			119.80		
			85.30			117.30		
			84.60			119.80		
			84.00			119.30		
			84.40	82.35	.521	120.80	119.40	.389
Air Dry	Shaker	15 sec.....	83.05			109.53		
			85.30			116.07		
			88.10			119.10		
			87.70			112.05		
			85.20	85.87	.622	121.69	115.68	1.452
Air Dry	Spatula	30 sec.....	87.60			113.20		
			73.10			118.20		
			73.90			123.00		
			75.10			121.80		
			74.30	76.80	1.835	120.00	119.24	1.163
Air Dry	Spatula	15 sec.....	70.90			116.90		
			75.82			115.10		
			83.83			113.80		
			72.57			104.80		
			78.50	76.32	1.542	117.00	113.72	1.516
Moist Soil	Shaker	30 sec.....	94.00			124.30		
			94.80			119.20		
			94.90			118.20		
			95.60			126.50		
			91.90	94.24	.428	124.30	122.50	1.085
Moist Soil	Shaker	15 sec.....	93.75			124.20		
			92.10			121.00		
			93.20			124.80		
			91.70			116.70		
			92.60	92.67	.248	122.70	121.88	.978
Moist Soil	Spatula	30 sec.....	93.90			116.00		
			94.40			122.00		
			100.40			120.15		
			97.90			113.50		
			96.60	96.64	.804	117.80	117.89	1.010
Moist Soil	Spatula	15 sec.....	76.70			127.30		
			65.60			118.50		
			76.50			122.10		
			74.80			119.60		
			84.00	75.52	1.975	120.90	121.68	1.032

While the data in the foregoing table are not entirely free from slight discrepancies, the following features are apparent.

1. Mixing the organic matter with the soil for 30 seconds shows a larger amount of ammonia accumulated than where the mixing occupied only 15 seconds.

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2. The experimental error of the average for 30-second mixings is smaller than that obtained for 15-second mixing periods.

3. In general, there is not as great a difference between the two periods of mixing with the shaker as is the case where the spatula is used.

4. The averages obtained with the shaker are larger than where the spatula was used to stir the organic matter into the soil.

5. A comparison of the moist and the air-dry soil shows a larger accumulation of ammonia in the case of the former soil. Since the moist soil was prepared by adding water to the dry soil a few minutes before mixing, there should exist no legitimate differences in the soil flora to account for this difference. The more plausible explanation would be a better oxygen pressure in moist soil. The moist soils were uniformly compacted by placing a 500-gm. weight on the surface of the moist soil in the tumbler, after the organic matter had been admixed.

6. In general, the experimental error of the averages of the soils prepared in the shaker is smaller than that obtained with the spatula, which bespeaks a narrower variation in the duplicate determinations.

This device is also exceptionally well adapted to the incorporation of very small quantities of materials of a finely pulverized condition of the soil. Although not tabulated here, additional data concerning the incorporation of lime, fertilization, et cetera, has been obtained, indicating the superiority of this method over the spatula method so widely recommended.

The shaker is easily sterilized and by rinsing it out with HCHO or C_2H_5OH it can be used for carrying out research which precludes contamination.

Many other sources of error in bacteriological work are under investigation, and it is hoped cognizance may be taken of this preliminary data, which have proven of value both to ourselves and other workers in this laboratory, and that it may act as a stimulus for an improved bacteriological technique in further biological work.

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STUDIES ON THE ACTIVITY OF SOIL PROTOZOA[1]

By

GEORGE P. KOCH, *Research Fellow, Rutgers College*

The possibility of soil protozoa acting as a destructive agent in soil fertility still seems to be an unsettled question.

In the most recent publication by Goodey (4) in which he employed old soils that were stored for some years, some of which showed the absence of protozoa, developed in hay infusion, he concluded as follows: "The results of the experiments described above, lead me to the conclusion, that the protozoa, including cilicates, amebae, and flagellates added to the soil have not been able to act as a factor limiting bacterial activity in the soil. Inferentially, therefore, the ciliates, amebae and flagellates obtainable from ordinary soil under cultural conditions do not function as the limiting factor. This is in accord with and extends the conclusion put forward in my earlier paper (3), that the ciliated protozoa are present in soil in an encysted condition and cannot function, therefore, as the factor limiting bacterial activity."

Russell (12) in his criticisms of Goodey's researches is still of the opinion that the results of his previous work (14,15) point to a factor which limits bacterial activities. He states, "The soil is known to be inhabited by numbers of bacteria, eel worms, vermes and numerous other organisms of higher orders and visible dimensions, and these lead active lives. Recently it has been shown in the Rothamsted Laboratory that a protozoan fauna also exists, some members at least of which are leading a trophic life." He (Russell) objects to the technique employed by Goodey in that, "The organisms inoculated into the soil are in the main those which figure largely in cultures made by adding soil to hay infusions. It has already been shown, however, (*v. supra*) that the culture fauna is distinct from the trophic soil fauna. There is, therefore, no evidence that the normal soil fauna was put back into the soil partially sterilized. On the contrary it apparently was not, nor is there evidence, except perhaps in one case, that the added organisms survived at all." * * * * "The protozoa are not obtained in pure culture alone, but they are added along with hay infusion and bacteria. Until more is known of the kind of protozoa occurring in the trophic state in the soil and their life history in the soil, it will be impossible to lay much stress on the negative results of re-infections."

[1] Received for publication May 31, 1916.

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In a recent work, on soil protozoa, Waksman (19) summarizes in part as follows: "Soil protozoa do not have any appreciable influence upon the ammonification by bacteria. The presence of protozoa acts detrimentally upon bacterial numbers so that when the conditions become favorable for protozoa development, the bacterial numbers decrease."

With the exception of examining and finding active protozoa present after the incubation period, this work of Waksman's is subject to the criticisms offered by Russell of Goodey's research. In no case were protozoa free from bacteria and decomposition products of organisms in the soil extract used as the inoculum into the sterile soil when the destructive effect of the protozoa was to be noted. Sherman (17, 18) in his researches with soil protozoa also realized this criticism to his work. Moreover, it would hardly seem scientifically accurate to compare the ammonifying power of organisms of the soil (bacteria and actinomyces in the main) from a depth of 20 inches which were originally entirely under anaerobic conditions, with organisms from the surface soil, as was undertaken by Waksman. It would be expected that the aerobic organisms contained in the upper layer of soil would be much more vigorous.

It is generally known that the ammonifying power of a soil, varies as the percentage of moisture which it contains. The production of ammonia by a soil flora usually increases until the moisture content equivalent to the physical optimum is reached. With heavier soils, the maximum is slightly higher than the physical optimum. Soon after the optimum condition of moisture, where there is maximum ammonification, has been reached, there is an abrupt decrease in ammonifying power. This is explained by the fact that the conditions become unfavorable for decomposition, and thus the processes proceed very slowly under these conditions which approach the anaerobic state. A parallel instance to this might be seen in the above cited work. There is reason to believe that the bacteria which were introduced with the protozoa in the protozoa culture were better ammonifiers than the bacteria free from protozoa, hence the protozoa might have been the cause of considerable destruction of bacteria and still an increase in ammonia would be found, being probably due to bacteria alone, as is recorded with soil B with 14 and 28 per cent moisture (protozoa plus bacteria) (19, p. 144).

In a previous publication the writer (7) gives an account of studies in the length of time of excystation of soil protozoa and some of the factors which influence the presence of protozoa in the active state in the soil. He also examined field and greenhouse soils to get some idea as to the extent of the activity of the protozoa present. For several reasons which need not be mentioned here, the effect of the presence of organic matter and the physical property of the soil upon the presence of protozoa in soils, in the active state, could not be worked out and included in the last publication, consequently this paper was prepared for this reason in part.

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Protozoan Activity in the Soil

The several methods used in studying protozoan activity in the soil were previously reviewed, hence no further mention will be made of them at this time.

Inasmuch as there were no extended data available which indicated the approximate time required for protozoa to excyst when the cysts come in contact with free water, the previous work (7) was carried out by making several examinations of the soils in question, for a period of two minutes only, thus eliminating the possible errors which might arise from the fact that the protective cysts might burst open during the examination and hence a false impression as to the real number of active protozoa in the soil would be obtained, as was suggested by Martin and Lewin (10). The writer realized that two minutes was a short period of time for a single examination, but when the field and greenhouse soils were examined for active protozoa for the actual length of time that the soil was under observation and in consideration of the number of samples taken, which was 14 or 15, it would seem that the active protozoa could have been detected and no doubt were, as will be demonstrated later. After making several thousand determinations, some of which were recorded in the last publication, to which reference has been made, and not finding any protozoa to excyst in 2 minutes, the 5 to 7 minute examination period was tried out and found to be satisfactory, as stated before (7, p. 486).

In the problems presented in this work, the direct examination of a small portion of soil was made for 5 minutes. Each soil was subjected to an examination extending from 15 to 20 minutes, i. e. 3 or 4 samples of each soil were taken. The low and the high power (4 mm.) of the microscope were employed. It was found that very great care must be exercised in employing the high power in determining active protozoa. There is still a chance for the investigator to err by employing the high power especially if there are protozoa which are as small as bacteria and have the Brownian movement, for in such a case it would be impossible to distinguish between protozoa and bacteria by this method. By extended examinations, it was found, however, with the soils employed, that practically all of the active protozoa could be seen with the low power.

In order to collect more data with regard to the influence of moisture content, physical character of the soil and the presence of organic matter upon the activity of protozoa in the active condition in the soil, 100-gm. portions of a typical Sassafras sandy loam soil which had been air dried, were weighed out into tumblers. The water-holding capacity of this soil was determined by the funnel method, which consists of merely determining the amount of water which is held by a weighed quantity of soil. It was found that 40 per cent of the amount of water held in this way by the soil, very closely approximated the physical optimum of the soil, deter-

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TABLE I

PRESENCE OF ACTIVE PROTOZOA IN A SASSAFRAS SOIL, WHICH HAD RECEIVED TREATMENTS OF DRIED BLOOD AND COW MANURE, AT VARIOUS MOISTURE CONTENTS AND AT DIFFERENT INTERVALS FOR A PERIOD OF 28 DAYS

Lab. No.	Kind of Soil	Material added	Amt. material added. gm.	Moisture added		Number of protozoa actually found per 0.33 gm. soil									
				Degree opti'um	c.c.	Days									
						1st	2nd	3rd	4th	6th	8th	10th	14th	21st	28th
21	Sassafras	Nothing	....	¾	13.95	....	....	2 F.[1]	....	....	....	....	....	....	....
22	"	"	....	1	18.60	....	....	....	1 F.	....	....	....	1 S.C.	....	....
23	"	"	....	1½	27.90	....	....	1 F. 1 S.C.	1 F.	....	....	....	1 S.C.	....	....
24	"	"	....	1¾	32.55	....	1 F.	....	2 F. 2 S.C.	1 F.	....	1 S.C.	1 S.C.	3 S.C. 1 L.C	1 F. 1 S.C.
31	"	Dried blood	2.85	¾	16.08	....	....	1 F.	2 F.	1 F.	....	....	....	....	....
32	"	"	2.85	1	21.45	....	....	2 F.	1 S.C.	1 F.	....	1 F.	....	....	....
33	"	"	2.85	1½	30.75	....	1 F. 1 S.C.	2 F. 1 S.C.	....	1 F. 1 S.C.	1 F. 1 S.C.	18 F.	....	....	....
34	"	"	2.85	1¾	35.40	....	4 F.	3 F.	1 F.	3 F. 3 S.C.	....	....	1 F.	1 S.C.	1 S.C.
41	"	Cow manure	2.85	¾	16.62	....	2 F.	3 F.	1 F.	2 F.	....	....	....	....	....
42	"	"	2.85	1	22.16	2 F.	3 F.	5 F.	2 F. 1 S.C.	1 F.	....	....	1 F.	....	....
43	"	"	2.85	1½	33.24	1 F.	1 F.	3 F.	9 F. 2 S.C.	1 F.	1 F. 4 S.C.	2 F. 5 S.C. 4 L.C.	....	....	1 F.
44	"	"	2.85	1¾	38.78	2 F.	1 F.	6 F. 1 S.C.	16 F. 3 S.C.	2 F.	....	1 F.	....	....	1 F. 1 S.C.
52	"	Sand	30	¾	12.07	....	....	....	....	....	....	2 F.	....	1 F.	....
62	"	"	30	1	16.10	....	....	....	....	....	....	....	....	....	....
72	"	"	30	1½	24.15	....	....	....	2 F.	....	1 S.C.	1 F.	1 F.	....	....
82	"	"	30	1¾	28.17	....	....	....	3 S.C.	3 F.	3 S.C.	1 F. 2 S.C.	1 F. 1 S.C.	1 S.C.	....

[1] F.—Flagellates. S. C.—Small Ciliates. L. C.—Large Ciliates.

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mined by means of feeling the soil. To each of four 100-gm. quantities of soil, seived air dry, 2.85 gm. of dried blood (representing 50 tons per acre on the basis of 2,700,000 pounds per acre-foot) were added. To another series of 4 tumblers, 2.85 gm. of dry manure, which had been seived through a 20-mesh seive, were added. Into each of 4 tumblers, 30-gm. quantities of pure washed quartz sand which passed a 40-mesh seive were weighed. Then 70 gm. of Sassafras soil were added to each portion. These tumblers containing the mixture of sand and soil, represented the third series. A fourth series of 4 tumblers with 100-gm. portions of soil received no treatment. Each of the soils with the several treatments was then thoroughly mixed by shaking the mixture in a small hard milk-shaking cup[1]. Then to each series, sterile water was added to an amount, making the content of one quantity 30 per cent of the water holding capacity, or three-fourths of the physical optimum, a second received sufficient to bring the moisture to 40 per cent of the water-holding capacity or one optimum, a third 60 per cent, and a fourth 70 per cent of the water-holding capacity. The soil was very carefully mixed so that the moisture would be as evenly distributed throughout the mass as possible. This was accomplished by emptying the contents of the tumblers on a large sheet of manila paper and mixing the material by passing it through the hands several times. Having replaced the contents in each tumbler and weighed the same, they were allowed to incubate at room temperature for a period of 28 days. Examinations of these soils for active protozoa were made by the method given above (three to four 5-minute examinations of each with the high and the low power of the microscope) on the 1st, 2nd, 3rd, 4th, 6th, 8th, 10th, 14th, 21st and 28th days after moistening the mixture. Before each examination was made the tumbler with its contents was carefully weighed and the moisture loss due to evaporation was restored. Then after the examination was complete, the sample was again weighed. By this method the amount of soil used for each examination was easily determined. Hence by counting the number of protozoa actually found in the examination, as was done, the number of active organisms (protozoa) per gram of soil could thus be calculated.

From examining Table I, it is apparent that the period of excystation of protozoa varies with the treatment of the soil, as in all cases where the soil received applications of cow manure the protozoa excysted sooner than they did in the other treatments. In all but one case, where the soil plus manure had a moisture content of three-fourths of the optimum, they were present in the active state on the first day after inoculation, while with no other treatments were they present on this day. Many more active protozoa were counted in the mixtures which received or-

[1] This was recommended by H. C. Lint. See "Sources of Error in Soil Bacteriological Analysis" by Lint, H. C., and Coleman, D. A., p. 157.

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ganic matter than where none was applied, likewise, the numbers were greatest in those to which the greatest amounts of moisture were added.

Increasing the porosity of this soil by means of sand did not seem to increase the number of active protozoa present, as there were less noted in the mixture of soil and sand than in the samples of soil alone.

It is noted in this problem, as was very apparent in the development of protozoa in cultural solutions (6), that the flagellates excyst first, the maximum number being present on the 3rd, 4th and 5th days. "At all temperatures, the flagellates develop sooner than the ciliates" (6, p. 557). It would thus appear that it requires a longer period of time for protozoa to excyst than is generally thought. Again, the active flagellates become less in number after the 6th day, when the ciliates become more numerous, so that on the 21st day only one small flagellate was noted in one soil while ciliates were found to be present in three. It is very plain that after a certain period of time the active protozoa decrease in number very rapidly, so that even though the moisture conditions would be considered very favorable and also an abundance of organic matter would be present, after a time the protozoan population in the active state would be almost *nil*. This above-mentioned fact no doubt explains the questions which Waksman (19, p. 136) raised in regard to the author's previous work[1] when he said, "The error made in limiting the time of examination to only 2 minutes is made clear by the fact that out of 20 greenhouse soils, where Koch found protozoa, the organisms recorded are in 5 cases ciliates and amoebae, and only in one case flagellates, and further as was shown by Sherman (16), Cunningham (2), by the writer and others, the number of flagellates is much larger than the number of ciliates, and we would expect that under conditions when moisture is the limiting factor, the smaller organisms would be able to lead an active life at a lower moisture content than the larger ones." It would seem, as shown above, that after a certain time and stage of decomposition of organic matter, active flagellates cease to be present to any great extent while living ciliates are more numerous. No doubt if the greenhouse soils to which Waksman refers had been examined soon after they were composted, the active flagellates would have out-numbered the ciliates considerably.

The question may be asked why more active protozoa were not found in the laboratory studies with the three soils in the previous work (7). This is explained by the fact that two of these soils were of close texture (heavy soils) one being a shale compost and the other a clay loam. It will be seen later that with a heavy soil (Penn clay loam) very few active protozoa are found. The first of these contained organic matter but as it had reached an advanced stage of decomposition it would not influence protozoan development to a very great extent. One small cili-

[1] It is presumed that Waksman refers to Koch's (7) "Activity of Soil Protozoa."

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ate was noted on the third day in the case of the field soil containing one and one-half optimum moisture. None of these soils received any organic matter in the laboratory treatments. No doubt their absence in the air-dried samples and those containing only one-half of the optimum of moisture was due to the lack of moisture. Furthermore even with a 15 to 20-minute examination, but very few (4 organisms) were found to be present in the examinations up to and including the 8th day of the present work, as shown in Table I (soil samples with one optimum and one and one-half optimums of moisture and receiving no organic matter, No. 22 and No. 23).

In order to demonstrate that the writer did not err in his 2-minute examination for 30 minutes in his previous work (7) as was questioned in the quotation above, after having found active protozoa to be present on the second day in this experiment, he concluded that certainly some would be present in the active condition on the third day. Hence to check up the 2-minute examination period, the length of time required to find a living protozoan was recorded on the third day. After the protozoa had been found, a different sample was taken and the examination of each soil was continued for a period of 20 minutes.

By noting the time required to find the active protozoa in the examination, it is seen that there are but few cases where these organisms were not found within the 2-minute period. Moreover, the samples of field and of greenhouse soil which were examined in the above referred work, were under observation for a 30-minute period, in which the experimenter is very liable to find the organisms. It is true that there were a comparatively large number of active protozoa present in the samples to which organic matter (dried blood and cow manure) had been added when this examination was made, but it will be noted that they were also found in the specified time (2 minutes) in the samples which received no organic matter, where the numbers were very low.

It might be stated that about 0.33 gm. of soil represented the amount taken for each daily examination for active protozoa, hence by multiplying by three the number of active protozoa recorded, found in Table I and in Table III, one could obtain a fairly good representation of the number of motile protozoa present per gram of soil. It would hardly seem that 0.33 gm. would be an adequate representative amount of soil upon which to base conclusions, but by this method (direct examination) it is, not possible to examine between the particles of more than this amount in 3 or 4 examinations. Moreover, in bacteriological technique, it is a common practice to pour plates from dilutions, which represent 1-10,000 and 1-50,000 of a gram. The largest number of flagellates found in one day's examination of a single sample was 18. This would mean that there were approximately 54 flagellates per gram of soil, which may be compared with at least 100,000,000 to 300,000,000 or more bacteria per gram of soil, as shown later.

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Having found that soil protozoa excyst for only a short period of time and that active flagellates appear first, followed by the ciliates and that the number of motile protozoa relative to bacteria is comparatively small, it was next desired to repeat the same experiment with a different soil.

TABLE II

THE LENGTH OF TIME REQUIRED TO FIND ACTIVE PROTOZOA AND THE NUMBERS FOUND BY THE DIRECT METHOD IN THE EXAMINATION OF THE VARIOUS TREATMENTS OF SASSAFRAS SOIL

Lab. No.	Organisms found in 20 minutes	Length of time to find organism min.-sec.
21	1 F.[1]	1:56
21	1 F.	3:10
22	none	....
23	1 F.	4:45
23	1 S.C.	1:46
24	none	....
31	1 F.	2:30
32	1 F.	1:40
	1 F.	0:30
33	1 F.	2:00
	1 F.	1:50
	1 S.C.	0:31
34	1 F.	1:00
	1 F.	0:26
	1 F.	0:40
41	1 F.	1:20
	1 F.	1:13
	1 F.	2:00
42	1 F.	2:50
	1 F.	3:10
	1 F.	2:05
42	1 F.	1:30
	1 S.C.	0:30
43	1 F.	2:28
	1 F.	0:32
	1 F.	1:19
44	1 F.	0:36
	1 F.	1:48
	1 F.	0:33
44	1 F.	1:03
	1 F.	0:09
44	1 F.	1:04
	1 S.C.	0:19
52	none	....
62	none	....
72	none	....
82	none	....

[1] F.—Flagellate. S. C.—Small Ciliates.

Hence a typical Penn clay loam was used. Inasmuch as active protozoa were found in all the samples of the Sassafras soil, even with lowest (three-fourths of the optimum) moisture content, in repeating the above problem with the Penn loam soil in each series, an additional quantity of soil was taken and moisture to the amount of one-third of the optimum was added.

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Likewise, the experiment was repeated with the Sassafras soil under the same conditions as in the preceding experiment, except that the moisture content was one-third of the optimum. Careful examinations for a period of 15 to 20 minutes were made of each sample on the 1st, 2nd, 3rd, 4th, 6th, 8th, 10th and 14th days.

Upon noting the upper portion of Table III and of Table I, it will be seen that the data indicate that with this soil (Sassafras) the moisture is the limiting factor which determines the presence of the protozoa in the active condition, while the organic matter and sand (porosity) added are secondary factors, a fact which did not hold for bacteria, in that some types of bacteria were always present at this (one-third of the optimum) moisture content. Again, with the Penn clay loam soil the greater number of protozoa became active on the 2nd, 3rd and 4th days, after which the number decreased so that there were practically none noted after the 6th day. With this soil, the number of active protozoa was very small as compared with the numbers counted in the previous problem (Table I) with the Sassafras soil. In many cases they were not found at all on any day, even where there was as much as 50 tons of cow manure per acre present, and with moisture to the amount of one and one-half optimum. With this in mind, it would hardly seem that they would play an important rôle in soil biological processes if their presence in the active state as determined by these methods, is used as an index. Apparently this type of soil (Penn loam) seemed to be a very unfavorable medium for active protozoa. A very small number might have excysted the second or third day, and so few being active for such a limited period of time, they might already have become inactive when the examinations were carried out. Dried blood seemed to favor their development to some extent. In sample No. 35, with the Penn soil containing 2.85 gm. of dried blood and a moisture content of one-third of the optimum, one small flagellate was seen to be present on the second day. This might have been due to the fact that the soil moisture was not equally distributed throughout the mixture as it is very difficult to get the moisture evenly distribued throughout the mass especially where such a small amount is added.

The addition of a sand to the soils increasing the porosity and aeration did not seem to encourage the increase of active protozoa.

In order to secure more data upon the effect of the addition of organic matter upon the presence of protozoa in the active condition, sample No. 22 with optimum moisture as shown in Table I, which had been under examination until after the 28th day, was employed for this work. In like manner, tumbler No. 20, which contained Sassafras soil with a moisture content of one-third optimum and tumblers No. 25 and No. 27 with Penn soil having moisture of one-third optimum and optimum, respectively, and which had been examined for 14 days were also used. To each of these soils, 2.85 gm. of dried blood were added and carefully

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TABLE III

PRESENCE OF ACTIVE PROTOZOA IN A SASSAFRAS SOIL AND PENN CLAY LOAM, WHICH HAD RECEIVED TREATMENTS OF DRIED BLOOD AND COW MANURE, AT VARIOUS MOISTURE CONTENTS AND AT DIFFERENT INTERVALS FOR A PERIOD OF 14 DAYS

Lab. No.	Kind of Soil	Material added	Amount of material added	Moisture added		Number of protozoa actually found after inoculation per 0.33 gm. soil							
				Degree opt'um	c.c.	Days							
						1st	2nd	3rd	4th	6th	8th	10th	14th
20	Sassafras	Nothing	....	1/3	6.20	.....	.....	.....	.....	.....	.....	.....	.....
30	"	Dried blood	2.85	1/3	7.15	.....	.....	.....	.....	.....	.....	.....	.....
40	"	Cow manure	2.85	1/3	7.38	.....	.....	.....	.....	.....	.....	.....	.....
50	"	Sand	30	1/3	5.37	.....	.....	.....	.....	.....	.....	.....	.....
25	Penn	Nothing	....	1/3	8.19	.....	.....	.....	.....	.....	.....	.....	.....
26	"	"	....	¾	18.42	.....	.....	.....	.....	.....	.....	.....	.....
27	"	"	....	1	24.56	.....	.....	.....	.....	.....	.....	.....	.....
28	"	"	....	1½	36.84	.....	.....	.....	.....	.....	.....	.....	.....
29	"	"	....	1¾	42.98	.....	2 F.[1]	.....	1 F.	.....	.....	.....	.....
35	"	Dried blood	2.85	1/3	9.14	.....	1 F.	.....	.....	.....	.....	.....	.....
36	"	"	2.85	¾	20.55	.....	1 F.	.....	.....	.....	.....	.....	.....
37	"	"	2.85	1	27.41	.....	.....	.....	.....	.....	.....	.....	.....
38	"	"	2.85	1½	39.69	.....	1 F. 1 S.C.	.....	.....	1 F.	.....	.....	.....
39	"	"	2.85	1¾	45.83	.....	1 F.	1 F.	1 S.C.	.....	.....	.....	.....
45	"	Cow manure	2.85	1/3	8.72	.....	.....	.....	.....	.....	.....	.....	.....
46	"	"	2.85	¾	19.62	.....	.....	.....	.....	.....	.....	.....	.....
47	"	"	2.85	1	26.16	.....	.....	.....	.....	.....	.....	.....	.....
48	"	"	2.85	1½	39.24	.....	.....	.....	.....	.....	.....	.....	.....
49	"	"	2.85	1¾	45.78	.....	1 F.	1 F. 1 S.C.	.....	.....	.....	.....	.....
53	"	Sand	2.85	1/3	6.15	.....	.....	.....	.....	.....	.....	.....	.....
54	"	"	2.85	¾	13.83	.....	.....	.....	.....	.....	.....	.....	.....
64	"	"	2.85	1	18.44	.....	.....	.....	.....	.....	.....	.....	.....
74	"	"	2.85	1½	27.66	.....	1 S.C.	.....	1 F.	.....	.....	.....	.....
84	"	"	2.85	1¾	32.27	.....	.....	.....	.....	.....	.....	.....	.....

[1] F.—Flagellates. S. C.—Small Ciliates.

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mixed as before. These samples were examined for living protozoa on the 1st, 2nd, 3rd, 4th, 6th, 8th and 14th days.

TABLE IV

PRESENCE OF ACTIVE PROTOZOA IN SOILS, WITH DIFFERENT MOISTURE CONTENTS, WHICH HAD PREVIOUSLY BEEN INCUBATED FOR A DEFINITE LENGTH OF TIME AND WHICH LATER RECEIVED ORGANIC MATTER

Lab. No.	Kind of Soil	Moisture during first incubation c.c.	Moisture after Dried Blood was added c.c.	Presence of active protozoa before Dried Blood was added	Presence of protozoa after inoculation							
					Days							
					1st	2nd	3rd	4th	6th	8th	10th	14th
20	Sass.	6.20	7.15	....	....	....	....	....	....	....	....	....
22	Sass.	18.60	21.45	....	....	....	....	....	....	....	....	....
25	Penn	8.19	9.14	....	....	....	....	....	....	....	....	....
27	Penn	24.56	27.41	....	....	....	....	....	....	....	....	....

From the above data it is seen that not only did the protozoa fail to excyst in the soils with a low moisture content, but they were not even noted in the soils which had an optimum moisture content. This might be explained by the fact that during the previous incubation of these soils, before the dried blood had been added, the organisms (bacteria, actinomyces, fungi, and in some cases protozoa, No. 22) multiplied, and decomposing more or less of the substances used for life processes, left products of assimilation behind which no doubt were unfavorable to their presence. Hence they did not appear in the sample even where the conditions were favorable for their development before. This shows that there is still another factor, other than moisture, presence of organic matter, physical property of the soil and temperature, which determines the presence or absence of active protozoa.

It might be stated that the inconsistency (as termed by Waksman) of Koch's (7) former results with the greenhouse soils, in that in some cases, active protozoa were found to be present at a lower moisture content than at others, and that Koch stated that the moisture was the primary limiting factor, can be explained as follows: By noting in the table the extent of protozoan activity in greenhouse soils (7) it will be seen that "no mixtures" and "20 per cent compost plus sand" contained the lowest percentage of moisture while the 40 per cent composts contained the highest. Thus when active protozoa were not found in soils with 34 to 36 per cent moisture, one of the reasons might have been that the actual free water at such a high compost was less than in a "no mixture" containing 22.59 per cent of moisture where motile protozoa were found.

The data as presented in Table I and II point to the fact that the greatest number of protozoa became active on the 2nd, 3rd, 4th and 6th

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TABLE V

PRESENCE OF ACTIVE PROTOZOA AND NUMBERS OF BACTERIA IN SAMPLES OF SASSAFRAS AND PENN CLAY LOAM SOILS WITH AN ADDITION OF DRIED BLOOD, AT DIFFERENT MOISTURE CONTENTS AND AT VARIOUS INTERVALS FOR A PERIOD OF 14 DAYS

Lab. No.	Kind of Soil	Moisture added c.c.	Days																							
			1st			2nd			3rd			4th			6th			8th			10th			14th		
			Protozoa	Bacteria	Av. Bact.	Protozoa	Bacteria	Av. Bact.	Protozoa	Bacteria	Av. Bact.	Protozoa	Bacteria	Av. Bact.	Protozoa	Bacteria	Av. Bact.	Protozoa	Bacteria	Av. Bact.	Protozoa	Bacteria	Av. Bact.	Protozoa	Bacteria	Av. Bact.
				15			44			104			*			115			168			174			*	
101	Sass.	7.15	—	11	12.0	—	54	50.0	—	90	101.6	—	132	117.5	—	108	112.0	—	120	150.0	—	168	148.0	—	36	38.0
				10			52			111			103			113			162			102			40	
				8			46			66			122			90			114			180			23	
102	"	7.15	—	11	9.6	—	39	45.0	—	103	86.6	—	102	112.0	—	90	98.0	—	108	100.0	—	90	118.0	—	29	24.6
				10			50			91			112			114			78			84			22	
				16			132			126			138			330			540			18			30	
103	"	21.45	—	18	17.6	—	96	108.0	1 F.	114	140.0	1 F. 1 S.C.	186	172.0	1 F.	242	275.0	1 S.C.	570	564.0	5 F.	12	12.0	—	28	26.6
				19			96			180			192			253			582			6			22	
				26			132			78			126			99			294			306			14	
104	"	21.45	—	16	17.0	—	162	140.0	1 F.	66	82.0	1 F.	120	116.0	—	231	183.3	—	342	314.0	1 F.	396	338.0	—	18	14.6
				9			126			102			102			220			306			312			12	
				20			33			29			29			42			48			6			43	
201	Penn	9.14	—	19	18.0	—	27	28.0	—	32	28.3	—	25	28.0	—	24	34.0	—	30	32.0	—	—	8.0	—	39	37.3
				15			24			24			30			36			18			18			30	
				22			32			29			25			24			30			12			38	
202	"	9.14	—	14	17.3	—	31	30.6	—	34	33.3	—	32	27.6	—	30	30.0	—	36	32.0	—	6	10.0	—	31	36.0
				16			29			37			26			36			30			12			39	
				64			108			36			102			66			48			30			90	
203	"	27.41	—	74	67.0	—	84	86.0	—	48	48.0	—	66	84.0	—	38	49.3	—	72	66.0	—	36	26.0	—	53	69.0
				63			66			60			84			44			78			12			64	
				53			72			96			66			33			174			90			102	
204	"	27.41	—	54	56.3	—	72	82.0	—	49	64.0	—	36	52.0	—	44	44.0	—	108	126.0	—	78	76.0	—	113	106.3
				62			102			47			54			55			96			60			104	

NOTE:—Numbers of bacteria represent millions per gram of soil. Numbers of protozoa are for 0.33 gm. of soil. * Lost.

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days. It was now desired to ascertain if during this period there would be a marked decrease in the number of bacteria. The possible destructive character of protozoa upon bacteria seemed to be the best index of the effects of protozoa. In the case where no protozoa were seen to be present, as at one-third of the optimum moisture content, one might infer that up to a certain point the bacterial multiplication would be consistent, while where the protozoa were seen to be present, their multiplication (bacteria) would be irregular. With this in mind four 200-gm. quantities of the same Sassafras loam and like quantities of Penn clay loam which had previously been used were weighed out into tumblers. Each tumbler received 5.70 gm. of dried blood, (or the equivalent of 50 tons per acre). Sufficient sterile water was added to two tumblers of the Sassafras series and two of the Penn series to make the soil plus dried blood of one-third of the optimum moisture content. In like manner two tumblers of each soil plus dried blood received sufficient water to bring the moisture content to optimum. These were carefully mixed as before and then placed in the incubator. Careful examinations of each tumbler for active protozoa for a period of 15 to 20 minutes were made from the 1st to the 14th days (inclusive) as before. The data were recorded each day. After the examinations had been completed 10 gm. were weighed out of each of the tumblers and this was used for making the bacteria counts[1].

Upon noting Table V it is seen that these data (part showing the presence of active protozoa) corroborate that which is shown in Tables I and III with these soils and the corresponding moisture content. Active protozoa were first seen to appear on the third day in the Sassafras soils containing the optimum amount of moisture. They were noted every day thereafter until and including the 10th with one sample, while with the other they were not seen on the 6th or 8th days. In the samples containing the lower amounts of moisture (one-third optimum) they were never seen to be present. Motile protozoa were never observed in any of the samples of the Penn clay loam soil. If they were active at any time in the samples of this soil they were either so minute that they could not be distinguished or the numbers were so very small that a 15 to 20 minute examination did not show any.

Is there any correlation between the bacterial numbers and the presence of active protozoa as determined by the direct examination? Glancing through Table V, which shows the number of bacteria present in the various samples of soil, it is seen that with samples No. 101 and No. 102, there is a more or less gradual increase in numbers of bacteria up to and

[1] The bacterial counts of each tumbler were made in triplicate on Lipman and Brown's "synthetic" agar. Lipman, J. G., and Brown, P. E. *In* N. J. Agr. Exp. Sta. 29th Ann. Rpt., p. 129-136. 1908.

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including the 10th day, and a very decided decrease on the 14th day. As stated before and shown in Table V, active protozoa were never seen to be present in either of these samples. In samples No. 103, there is a marked increase in bacterial numbers until the 8th day even though active protozoa were observed on the 4th, 6th and 8th days. On the 10th day there is a very marked decrease in bacteria and a greater number of protozoa were also counted on this day. In the case of sample No. 104, this increase in bacterial numbers from day to day is not so consistent as it is with No. 103 (the duplicate of 104), but even in the latter sample, we do not find any reduction in bacteria after the third day until the 14th even though motile protozoa were observed. If the active protozoa in No. 103 were effective in destroying bacteria, then the multiplication of bacteria must have been much greater than that in sample No. 104 in order that the greater number of bacteria in No. 103 can be accounted for, as more protozoa were also noted in this latter sample than in No. 104; or else the motile protozoa did not affect the numbers of bacteria to any appreciable extent, since it is noted that in samples Nos. 101 and 102, where the moisture content was low, there was a great decrease of bacterial numbers on the 14th day and yet living protozoa were never seen. Again, on the 14th day, in samples Nos. 103 and 104, we find a great decrease in bacterial numbers. Is this due to the effect of the active protozoa which were seen to be present on the 10th day? If this is true, it would agree with Russell (11) in his objection that the effect of the detrimental factor cannot be seen in a 7-day incubation period; hence the protozoa which had been present in the active condition might not have been of the destructive type. Again, might the decrease in the numbers of bacteria have been due to other causes, as the presence of fungi or other organisms, or to the presence of toxic decomposition products?

Upon examining the results, with the Penn soil, it is seen that in the samples containing moisture of 1/3 optimum, the bacterial numbers were practically constant until the 10th day. On this day a marked decrease was noted and then a great increase on the 14th day. It would hardly seem possible that this decrease in bacterial numbers was due to a detrimental factor (namely protozoa) as they were never observed to be present in the motile condition. The irregularity in numbers of bacteria was noted in a piece of work by the writer (8) where he counted organisms (*B. coli.*) developed from day to day over a period of time. Just such a cause may be due to the variation in the numbers of bacteria which were observed in this problem. Again in Nos. 203 and 204 there is a fluctuation in bacterial numbers from day to day and still no protozoa were ever noted to be present.

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It is true that an investigator could hardly conclude that the results of such a problem as above, where all the organisms of the soil as well as the other conditions as moisture, assimilation products, etc., must be considered, are due to the one specific factor with which he is working. However, the intention of this work, as in the case of the previous work (7), is to study normal soils as they occur and thus see whether or not protozoa if they are a factor, exist in the normal conditions (that is, as nearly normal as possible in carrying out laboratory experiments) and to correlate if possible the factors studied (bacteria and protozoa) under these conditions.

Can the results obtained by employing partially sterilized sewage-sick soils or partially sterile normal soils, in which cases only some of the factors are eliminated, be used to demonstrate the effects of a detrimental factor (probably protozoa) applied to normal agricultural soils? As shown recently by Coleman, Lint and Kopeloff (1), partial sterilization of normal soils destroys only some of the factors which function in soil fertility, and it greatly influences (alters) the character of the soil as a medium.

No doubt, under very abnormal conditions (as regards agricultural practices), for instance in the case of sewage-sick soils which contain a great abundance of organic matter and whose floral population is entirely different from that of soils under tillage, containing not only an abundance of protozoa but also eel worms, vermes and many other living forms, and under other conditions, as aeration, etc., entirely different from those under which the soil is tilled normally, a limiting factor might be of immense importance.

In the light of the results of this paper and of the paper cited above (7), it would seem that in a practical way, protozoa are of slight importance in limiting the bacterial flora of the soil, as their presence in the active state is very limited. In no cases were they found to be present in the active state in the field soils examined, in only 6 out of the 20 greenhouse soils were protozoa found, and in these soils their numbers were very limited. The data as given in this paper would indicate their presence in some soils to which a large amount of organic matter (50 tons per acre) has been applied, but even under these conditions of fertility, when more organic matter is applied than is ever used under ordinary field agricultural methods, they are active only to a very limited extent, the numbers decreasing decidedly 8 or 10 days after the organic matter has been added. Moisture seems to be the primary factor which in these soils and others, determines their presence in the active condition, as active protozoa were never noted in soils which were air-dry or had a very low moisture content, even though there was an abundance of organic matter and the texture was of very open character. This is not true of bacteria however, as some are in the active condition in air-dried soils.

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Separation of Bacteria from Protozoa

Under certain conditions, in carrying out soil biological experiments in the laboratory and eventually in the field (9) for a period of 7 to 10 days, we have protozoa present in the active condition. Are they an influential factor in the biological method as now employed? Considering the very small numbers which are present in the active condition as compared with bacteria, and the fact that they are present for such a short period of time, we would hardly think they would be a factor. However, in desiring to prove this point, the author is attempting to get a protozoa culture free from bacteria. He plans to inoculate the medium with these clean cultures and in this way note their effect upon bacterial activities.

For this work a special centrifuging tube was devised by the writer and has been proving satisfactory although a complete separation of bacteria from protozoa has not yet been accomplished.

The apparatus, as pictured in figure 1, consists of a glass centrifuging burette, 10½ inches long and having a capacity of 6 c.c. This special burette has two stop cocks at its lower extremity. The contents of the small tube between the stop cocks is about 1 c.c.

The protozoa which are much heavier than bacteria are forced down by gravity in the centrifuging process as has been previously tried by Russell and Golding (13) and later by Jones (5).

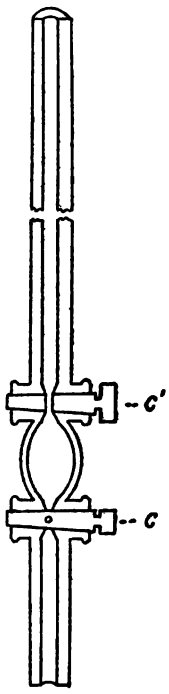

Fig. 1—Diagram of apparatus used for separating protozoa from bacteria.[1]

The procedure of this method is as follows: Cock C being closed and cock C′ open and the lower end (delivery tube) of the burette having been plugged with cotton, 5 c.c. of the medium containing protozoa and bacteria are introduced into the sterile apparatus. The apparatus with contents is now placed into the centrifuge and a certain number of revolutions are made. The protozoa are thus forced down through the opening of cock C′ and are held in the small tube between C and C′. Cock C′ is closed and the solution in the upper part of the burette which contains the greater number of bacteria is poured out. This section of the apparatus is now sterilized with alcohol and then some of the sterile medium is introduced. Cock C′ is now opened and the protozoa which are held in the tube between C and C′ are washed into this sterile medium. Centrifuging is repeated, cock C′ closed and the same process repeated as before. In this manner the protozoa can be washed several times until the bacteria are all washed from them. The protozoa having thus been freed from the bacteria, cock C is opened and the protozoa are delivered into the desired container by means of the small delivery tube at the lower extremity of the burette.

[1] The author is indebted to Dr. J. W. Shive for preparing this drawing.

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Summary

Under the conditions with which the problems recorded in this paper were carried out, the following results as to protozoan activity in the soils have been noted:

1. The direct examination of the soil under the microscope for a period of 15 to 20 minutes (taking 3 or 4 samples), gives a fair indication as to the relative extent of active protozoa in the soil.

2. Practically all of the protozoa as found in the soil can be observed under the low power of the microscope by the careful experimenter.

3. The addition of organic matter to the Sassafras sandy loam soil encouraged a greater protozoan development than where none had been added.

4. Additions of dried blood to the Penn clay loam soil increased the number of active protozoa but very little while cow manure had no effect on the protozoan activity of this soil.

5. Increasing the porosity and aeration of the soils by the addition of sand did not increase the number of motile protozoa.

6. In the Sassafras soil to which cow manure had been added, flagellates were noted on the 1st day, while with the other samples they were not seen until the 2nd or 3rd and in some cases the 4th day. The maximum numbers were recorded on the 4th and 6th days after which there was a marked decrease so that on the 21st and 28th days practically none were found to be in the active state. This would indicate that the destructive ability, (if soil protozoa possesses that factor) would be present for only a limited period, namely the early stages of organic decomposition.

7. Flagellates were the first protozoa to excyst, later, on the 3rd and 4th days, small ciliates appeared. On the 14th, 21st and 28th days, small ciliates were more numerous than flagellates.

8. With these soils, the largest number of protozoa was recorded where the moisture was highest.

9. The Penn clay loam soil seemed to be a very undesirable medium for protozoa, as very few of these organisms were noted in any of the samples compared with those found in the Sassafras soil.

10. At the lowest moisture content (one-third of the optimum) protozoa did not become active.

11. With these soils, moisture seemed to be the primary limiting factor which determined the presence or absence of active protozoa.

12. With one exception, no correlation between the presence of protozoa in the active condition and numbers of bacteria could be seen. Increased numbers of bacteria were observed irrespective of the presence or absence of living protozoa.

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13. On the 14th day in the soils, even where no protozoa were found in the active condition, there was a great decrease in bacterial numbers.

14. Inasmuch as the numbers of protozoa in comparison with the bacterial numbers are so small, even in the presence of such abnormal quantities of organic matter as were used in these experiments, it hardly seems that they would be of very great importance in agricultural practices.

It has been previously shown (7):

1. Under ordinary greenhouse conditions, small ciliates, flagellates and amoebae are active in some soils, but their presence is very limited.

2. Active protozoa (small ciliates, large ciliates, flagellates, and amoebae) do not seem to be present in field soils with a normal moisture content, and even when the moisture content is slightly supernormal; hence, they would not be a limiting factor in the soil.

Literature Cited

(1) Coleman, D. A., Lint, H. C., and Kopeloff, N.
1916. Can soil be sterilized without radical alteration? *In* Soil Sci., v. 1, no. 3, p. 258-274.

(2) Cunningham, Andrew.
1915. Studies on soil protozoa. *In* Jour. Agr. Sci., v. 7, pt. 1, p. 49-74.

(3) Goodey, T.
1911. A contribution to our knowledge of the protozoa of the soil. *In* Proc. Roy. Soc. (London), S. B., v. 84, no. 570, p. 165-180. 1 fig., pl. 4.

(4) Goodey, T.
1915. Investigations on protozoa in relation to the factor limiting bacterial activity in soil. *In* Proc. Roy. Soc. (London), S. B., v. 88, no. B. 606, p. 437-456.

(5) Jones, Henry N.
1916. A simple method for the elimination of protozoa from mixed cultures of bacteria. *In* Science, n. s., v. 43, no. 1098, p. 68-69.

(6) Koch, Geo. P.
1915. Soil Protozoa. *In* Jour. Agr. Research, v. 4, no. 6, p. 511-559.

(7) Koch, Geo. P.
1915. Activity of soil protozoa. *In* Jour. Agr. Research, v. 5, no. 11, p. 477-488.

(8) Koch, Geo. P.
1916. Diatase activity and invertase activity of bacteria. *In* Soil Sci., v. 1, no. 2, p. 179-196.

(9) Koch, Geo. P.
Preliminary investigations in comparison of field with laboratory experiments in soil biology. *In* Soil Sci., v. 2, no. 1, p. 87-92.

(10) Martin, C. H., and Lewin, K. R.
1915. Notes on some methods for the examination of soil protozoa. *In* Jour. Agr. Sci., v. 7, pt. 1, p. 106-119, pl. 2, 3.

(11) Russell, E. J.
1913. The complexity of the microörganic population of the soil. *In* Science, n. s., v. 37, p. 519-522.

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(12) RUSSELL, E. J.
1915. Soil protozoa and soil bacteria. *In* Proc. Roy. Soc. (London), S. B., v. 89, no. B, 610, p. 76-82.
(13) RUSSELL, E. J., and GOLDING, J.
1912. Investigations on "sickness" in soil. *In* Jour. Agr. Sci., v. 5, pt. 1, p. 27-47.
(14) RUSSELL, E. J., and HUTCHINSON, H. B.
1909. The effect of partial sterilization of soil in the production of plant food. *In* Jour. Agr. Sci., v. 3, pt. 2, p. 111-144, pl. 8, 9.
(15) RUSSELL, E. J., and HUTCHINSON, H. B.
1913. The effect of partial sterilization of soil on the production of plant food. II, The limitation of bacterial numbers in soils and its consequences. *In* Jour. Agr. Sci., v. 5, pt. 2, p. 152-221.
(16) SHERMAN, J. M.
1914. The number and growth of protozoa in the soil. *In* Centbl. Bakt. (etc.), Abt. 2, Bd. 11, no. 18-23, p. 625-630.
(17) SHERMAN, J. M.
1916. Studies on soil protozoa and their relation to the bacterial flora, I. *In* Jour. Bact., v. 1, no. 1, p. 35-66.
(18) SHERMAN, J. M.
1916. Studies in soil protozoa and their relation to the bacterial flora, II. *In* Jour. Bact., v. 1, no. 2, p. 165-185.
(19) WAKSMAN, S. A.
1916. Studies on soil protozoa. *In* Soil Sci., v. 1, no. 2, p. 135-152.

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AZOTOBACTER IN HAWAIIAN SOILS[1]

By

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Since those reading this brief paper are doubtless familiar with the aerobic, non-symbiotic species of nitrogen fixing bacteria termed Azotobacter, a detailed discussion of the properties of this class of microorganisms together with a review of its extremely copious literature would here be both objectionable and superfluous.

While working in the laboratories of Soil Chemistry and Bacteriology at the California Agricultural Experiment Station some time ago, many samples of soil from different parts of the world were examined by the writer for Azotobacter species (1). This work was done under the direction of Dr. C. B. Lipman and with the object of ascertaining the universality of distribution of this most interesting group of soil organisms. We were especially fortunate in having at our disposal the large and unusually complete soil collection of the late Dr. E. W. Hilgard, from which about fifty soils, from widely separated parts of the world, were chosen for the work. These included some six or eight samples of Hawaiian soils, and it may be added that later about a dozen more samples from the Hawaiian Islands were further investigated. With but one or two possible exceptions, no Azotobacter species were discovered in the Hawaiian soils examined. Were these dry soil samples too old to retain this species still in a living condition even had they been originally present, or had Azotobacter not yet become generally distributed in the soils of these isolated islands which, geologically speaking, are of very recent origin? These were questions which, at the time we were unable to answer, but which now furnish an excuse for the publication of this paper.

About six months ago it fell to the writer's lot to visit the four large islands of the Hawaiian group and to collect a large number of soil samples from the several typical soil areas there found. These samples were drawn for another purpose, but, remembering his previous failure to isolate Azotobacter from similar soils, several fresh samples from each of the islands of Oahu, Hawaii, Maui and Kauai were studied as to their

[1] Received for publication June 20, 1916.

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Azotobacter content. So far as the writer is aware, nothing has appeared concerning the forms or physiological activities of Azotobacter species isolated from Hawaiian soils.

The samples were all taken from cultivated fields which at the time were carrying heavy crops of sugar cane.

Experimental

The soil samples were all received in new, heavy-canvas, sterilized bags, each of which held about ten pounds of soil. A sterilized (in flame) trowel was used in taking the samples. As soon as a bag was filled it was closed and labeled. These small bags were placed in large sugar bags and shipped directly to the laboratory in Honolulu. When received, the different samples were carefully spread out on sterile (washed with alcohol) pieces of oil-cloth about 2 feet square, in the basement, and allowed to air-dry. The samples were then passed through sterile 2-mm. sieves. The soils were then replaced in their original bags and so kept in a large cold chamber until used. Over one hundred samples of surface soils were so collected and treated, together with their subsoils. Only thirty surface soils were used in the work here reported, however.

Table I gives the laboratory number and the localities from whence each of the different soil samples came.

In selecting these samples practically all of the different arable soil types of any considerable area encountered here on the islands have been covered. Where two samples were taken from the same plantation, the first was drawn from the low lands near the sea, while the other came from the higher fields (from 700 to 1200 feet above sea level).

I have on hand analytical data covering all of the above samples, but they find no place in this paper. It may be stated, however, that all of the soils listed above, with the exception of two or three coral formations, are laterite soils derived from basaltic lavas, and contain from 40 to 65 per cent combined oxides of iron and aluminum, "Hilgard Method of Analysis." Physically, most of the soils above listed would be classed as silty, clay loams of high organic matter content, the percentage of total nitrogen varying from 0.2 per cent to over 1.00 per cent. Most of the soils are of a dark brown or chocolate color, although there are some exceptions. Those from the Oahu Sugar Co. are a very bright brick red, while those from Honolulu Plantation are a light drab or gray.

The nutrient solution employed was made up as follows:

In 1000 c.c. tap water was dissolved:

0.2 gm. K_2HPO_4
0.2 gm. $MgSO_4$
0.5 gm. NaCl
20.0 gm. mannite
1 drop of a 10 per cent $FeCl_3$ solution.

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A few drops of phenolphthalein were then added to the solution and it was rendered slightly alkaline with a 5 per cent solution of KOH. One-hundred-c.c. portions of this solution were placed in 500-c.c. Jena Erlenmeyer flasks, plugged with cotton, and sterilized at 10 pounds pressure for 15 minutes. Each of these flasks, cooled, was then inoculated with exactly 1 gm. of the air-dried soil under investigation, the nitrogen contained therein being finally subtracted from the total fixation as shown in Table III.

TABLE I

LOCALITIES FROM WHENCE SOIL SAMPLES WERE TAKEN

Laboratory No.	Island	Plantation
1	Hawaii	Waiakea Mill Co.
2	Hawaii	Waiakea Mill Co.
3	Hawaii	Onomea Sugar Co.
4	Hawaii	Onomea Sugar Co.
5	Hawaii	Hawaiian Agricultural Co.
6	Hawaii	Hawaiian Agricultural Co.
7	Hawaii	Pacific Sugar Mill.
8	Hawaii	Pacific Sugar Mill.
9	Hawaii	Honokaa Sugar Co.
10	Hawaii	Honokaa Sugar Co.
11	Hawaii	Hamakua Mill Co.
12	Hawaii	Hamakua Mill Co.
13	Hawaii	Kaiwiki Sugar Co.
14	Hawaii	Kaiwiki Sugar Co.
15	Hawaii	Pepeekeo Sugar Co.
16	Hawaii	Kukaiau Plantation Co.
17	Oahu	Experiment Station, Honolulu.
18	Oahu	Honolulu Plantation Co.
19	Oahu	Honolulu Plantation Co.
20	Oahu	Ewa Plantation Co.
21	Oahu	Waipio Field Experiment Station.
22	Oahu	Oahu Sugar Co.
23	Oahu	Oahu Sugar Co.
24	Maui	Hawaiian Commercial and Sugar Co.
25	Maui	Hawaiian Commercial and Sugar Co.
26	Maui	Pioneer Mill Co.
27	Maui	Pioneer Mill Co.
28	Kauai	McBryde Sugar Co.
29	Kauai	McBryde Sugar Co.
30	Kauai	Hawaiian Sugar Co.

These mixed cultures were then incubated 3 weeks at 28° C. At the end of 5 days, beautiful Azotobacter membranes had appeared in many of the flasks. At the end of one week the appearance of these cultures was as follows:

SOIL CULTURE No. 1.

Color of solution	—	gray
Pigment	—	white, glistening
Membrane	—	at edges of flask only
Gas production	—	slight
Turbidity	—	slight
Odor	—	butyric acid, slight

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SOIL CULTURE No. 2.
Very similar to No. 1, except that membrane extended over entire surface.

SOIL CULTURE No. 3.

Color of solution	—	colorless
Pigment	—	none
Membrane	—	very thin, at edges
Gas production	—	heavy
Turbidity	—	slight
Odor	—	strong butyric

SOIL CULTURE No. 4.
Similar to No. 3, except that there was no membrane.

SOIL CULTURE No. 5.

Color of solution	—	drab
Pigment	—	heavy, orange-colored
Membrane	—	heavy over entire surface
Gas production	—	medium
Turbidity	—	heavy
Odor	—	butyric

SOIL CULTURE No. 6.

Color of solution	—	gray
Pigment	—	beautiful, white, glistening
Membrane	—	heavy over entire surface
Gas production	—	heavy
Turbidity	—	very turbid
Odor	—	vile, putrescent

This organism is apparently quite widely distributed throughout these island soils, for the description of Soil Culture No. 6 can very well serve for the following soil cultures:—Nos. 8, 10, 12, 16, 23, 26 and 29. It is true that they were not all absolutely identical in appearance, yet their remarkable similarity showed them to contain the same species of Azotobacter. This was verified by microscopical examination.

SOIL CULTURE No. 7.

Color of solution	—	brown
Pigment	—	none
Membrane	—	none
Gas production	—	heavy
Turbidity	—	heavy
Odor	—	strongly butyric

SOIL CULTURE No. 9.
Identical with Soil Culture No. 7.

SOIL CULTURE No. 11.

Color of solution	—	yellow
Pigment	—	golden yellow
Membrane	—	heavy over entire surface
Gas production	—	strong
Turbidity	—	medium
Odor	—	strong, butyric

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SOIL CULTURE No. 13.
Description omitted by mistake.

SOIL CULTURE No. 14.
Similar to Soil Culture No. 7.

SOIL CULTURE No. 15.
Identical with Soil Culture No. 11.

SOIL CULTURE No. 17.

Color of solution	—	drab to chocolate color
Pigment	—	black
Membrane	—	over entire surface, heavy
Gas production	—	heavy
Turbidity	—	medium
Odor	—	none

SOIL CULTURE No. 18.

Color of solution	—	light brown
Pigment	—	tan-colored, black spots
Membrane	—	over entire surface
Gas production	—	slight
Turbidity	—	medium
Odor	—	strong, butyric

SOIL CULTURE No. 19.

Color of solution	—	almost colorless
Pigment	—	black with dark orange spots
Membrane	—	heavy over entire surface
Gas production	—	medium
Turbidity	—	medium
Odor	—	damp leaf mold, earthy

SOIL CULTURE No. 20.

Color of solution	—	none
Pigment	—	white, glistening; slightly yellowish
Membrane	—	thick over entire surface
Gas production	—	slight
Turbidity	—	slight
Odor	—	butyric

SOIL CULTURE No. 21.

Color of solution	—	light brown
Pigment	—	smooth, white; black at edges
Membrane	—	very thin over entire surface
Gas production	—	none
Turbidity	—	slight
Odor	—	putrescent

SOIL CULTURE No. 22.

Color of solution	—	reddish
Pigment	—	black with orange spots
Membrane	—	thick, very mottled
Gas production	—	heavy
Turbidity	—	medium
Odor	—	weak butyric

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SOIL CULTURE No. 24.

Color of solution	—	yellowish green
Pigment	—	beautiful golden yellow
Membrane	—	over entire surface, heavy
Gas production	—	heavy
Turbidity	—	very turbid
Odor	—	strong butyric

SOIL CULTURE No. 25.

Similar to Soil Culture No. 24 except that here the membrane was very thin and at the edges of the flask only.

SOIL CULTURE No. 27.

No pigment and no membrane. Similar to Soil Culture No. 7.

SOIL CULTURE No. 28.

Very similar to Soil Culture No. 24.

SOIL CULTURE No. 30.

Color of solution	—	brownish
Pigment	—	pale orange-colored with black spots
Membrane	—	thin
Gas production	—	weak
Turbidity	—	slight
Odor	—	weakly putrescent

After recording the appearance of the mixed cultures, all of those showing membranes dissimilar in color or appearance were plated out on mannite agar and the plates incubated for from 4 to 8 days at 28° C. The writer will not burden the reader with a detailed description of these plates. Suffice it to say that, in the end (after several replatings), four different Azotobacter forms were isolated in pure cultures. Two of these were identified as *A. chroococcum* (isolated from Soil Culture No. 18), and *A. Vinelandii* (isolated from Soil Culture No. 24). Of the two remaining unidentified organisms, one was very similar to *A. Vinelandii* in morphology and cultural characteristics, with the following few exceptions. The individual cells are slightly smaller although of similar shape. There is no tendency to form a colored pigment. Even on old mannite agar slants the growth remains white, there being no yellow or orange tint observed. This organism is probably a variety of *A. Vinelandii* and was isolated from Soil Culture No. 16. This form was universally present in those original soil cultures described as showing a "beautiful, white, glistening membrane," and is apparently quite widely distributed in these island soils (see description of Soil Culture No. 6, above). The other unidentified member of this group is almost identical in appearance and in cultural characteristics with "B. 29 n. sp." derived from an Egyptian soil, a micro-photograph of which is to be seen in "Centralblatt für Bakteriologie," (etc.), Bd. 44, 1915, opposite page 511. A brief description of this species as found in the soil from the Hawaiian Commercial and Sugar Co., Puunene, Maui (Soil Culture No. 29) is as follows:

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Shape: Oval to bacillus shaped; rounded ends. Occur singly or in short chains.
Size: 1.4 μ wide by 2.6 to 2.8 μ long.
Staining Properties: Stains solidly with methylene blue or Bismark brown although the cell walls at the edge are usually a little darker. There are no apparent internal markings or granules as is the case with *A. Chroococcum.*
Motility: None.
Spores: None found even in old slant cultures.
Mannite agar slant: Narrow filiform; edges wavy and beaded; surface flat; color white, old cultures slightly brownish tint; moist growth, slightly raised at edges; slow grower.
Colonies on mannite agar: White to grayish; raised; round with slightly beaded edge; moist and glistening; no internal markings.
Potato Slant: Very slow thin growth.
Mannite agar stab culture: Very little growth in depths; spreading growth at surface.
Nitrogen-fixing power: In soils, 4.0 mg. per gm. of mannite; in solution, 6.0 mg. per gm. of mannite.

At the end of 3 weeks' incubation period, all of the mixed cultures were analyzed for total nitrogen by Hibbard's modified Gunning method (2), with the results as set forth in Table II.

TABLE II

NITROGEN FIXATION IN MANNITE SOLUTIONS BY 30 HAWAIIAN SOILS

Lab. No.	c.c. N/10 HCl used	N. found mg.	N. in 1 gm. Soil. mg.	N. fixed per culture. mg.	N. fixed per gm. mannite. mg.
1	11.4	16.0	8.7	7.3	3.65
2	17.9	25.1	10.0	15.1	7.55
3	9.1	12.7	5.2	7.5	3.75
4	8.4	11.8	5.4	6.4	3.20
5	13.5	18.9	3.5	15.4	7.70
6	13.8	19.3	3.9	15.4	7.70
7	8.4	11.8	4.1	7.7	3.85
8	13.7	19.2	6.2	13.0	6.50
9	9.8	13.7	6.0	7.7	3.85
10	10.4	14.6	4.0	10.6	5.30
11	9.9	13.9	4.3	9.6	4.80
12	11.3	15.8	7.4	8.4	4.20
13	12.8	17.9	7.4	10.5	5.25
14	9.3	13.0	5.3	7.7	3.85
15	10.6	14.8	5.7	9.1	4.55
16	22.5	31.5	4.2	27.3	13.65
17	15.6	21.8	1.8	20.0	10.00
18	14.8	20.7	3.3	17.4	8.70
19	12.4	17.4	1.3	16.1	8.05
20	9.3	13.0	0.7	12.3	6.15
21	11.8	16.5	1.5	15.0	7.50
22	10.4	14.6	1.7	12.9	6.45
23	11.9	16.7	2.2	14.5	7.25
24	9.5	13.3	1.0	12.3	6.15
25	4.2	5.9	1.0	4.9	2.45
26	6.0	8.4	1.9	6.5	3.25
27	6.0	8.4	2.1	6.3	3.15
28	10.7	15.0	3.1	11.9	5.95
29	7.4	10.4	2.7	7.7	3.85
30	7.8	10.9	1.7	9.2	4.60

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A few interesting facts are brought out in a study of this table. As was pointed out by Lipman and Burgess (1), there seems to be a relation, at least in many cases, between the percentage of nitrogen in a given soil and that soil's powers of non-symbiotic nitrogen fixation; the higher the total nitrogen content, the lower the fixation, and vice versa. As is well known, the soils on the island of Hawaii run far higher in total nitrogen than do the soils from any of the other islands, while, generally speaking, those of Oahu, except on the windward side, are among the lowest. A glance at Table II shows that, taken by and large, the first fifteen soils (from Hawaii) show a lower fixation as well as a much higher total nitrogen content than do the seven soils which come from Oahu. This, however, does not hold true for the samples from the islands of Maui and Kauai, where fixing powers are the lowest of all. The exception here apparently proves the rule, for Soil No. 16 from the Kukaiau Plantation, carrying over 0.4 per cent total nitrogen, gives us the highest fixation of all, i. e. 13.65 mg. of nitrogen per gram of mannite consumed.

Of the thirty soil samples included in this work but five appeared to be absolutely devoid of Azotobacter growth. These were Nos. 4, 7, 9, 14 and 27. In these cultures, nevertheless, some fixation is recorded which indicates that probably certain Clostridium forms are also fairly prevalent here. It is further worthy of mention that the fixation of nitrogen in these five cultures, although from widely separated districts, is practically identical (varying from 3.15 to 3.85 mg. only), indicating a common organism.

While the amounts of nitrogen fixed in these mixed solution cultures are in no case exceptionally high, they are, taken by and large, far larger than the writer had anticipated from his earlier work reported elsewhere.

The four species of Azotobacter above isolated and described were now examined regarding their abilities to fix nitrogen in pure cultures, both in solutions and in soils. Young mannite agar slants (3 to 4 days old) were used in making the bacterial suspensions. This was done by pouring 10 c.c. of sterile tap water over the growth on an agar slant and working it up with a sterile platinum needle. All of the suspensions were fairly cloudy in appearance. Amounts of 1 c.c. each of these were used to inoculate 100-c.c. portions of the above sterile mannite solution in 500-c.c. flasks. Other 1-c.c. portions were used to inoculate 100-gm. portions of the station soil in covered tumblers which had previously been sterilized for about 3 hours at 15 pounds, and had each received 2 gm. of mannite after being cooled. The solution cultures only were run in duplicate. After incubating 3 weeks at 28° C., total nitrogen determinations were made by the method used above with the results as set forth in Table III.

A study of this table reveals the interesting fact that, contrary to custom, more nitrogen was fixed by these organisms in solutions than in soils, with but the one exception, *A. Vinelandii,* n. var.? In this culture,

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however, a fine white mold growth was noticed toward the latter part of the incubation period which, to a certain extent, vitiates the result. Whether or not the soil used suffered so serious a change during sterilization as to be rendered unfit for Azotobacter growth, or whether these microörganisms work to better advantage in solutions than in soils, are questions which cannot now be answered. The pure cultures in solution all gave beautiful membranes. The first was light brown in color; the second, orange; the third, white; and the last, a very thin grayish film.

It was intended at the inception of this work to run all of the soils for nitrogen fixation by the "beaker method," in which the original soil plus mannite is used as its own culture medium, but, due to the press of other work, this has not been found possible. It was further intended to have micro-photographs taken of any unusual Azotobacter forms, should any be found, but, the station photographer being absent on a six months' vacation has caused us to abandon this also.

TABLE III

NITROGEN FIXATION BY PURE CULTURES OF AZOTOBACTER OBTAINED FROM HAWAIIAN SOILS; IN SOLUTIONS AND IN SOILS

SOLUTION CULTURES

Organism	c.c. N/10 HCl used	N. found per culture. mg.	N. fixed per gm. mannite. mg.	Average mg.
A. chroococcum, var...	6.8	9.5	4.75	4.62
A. chroococcum, var...	6.4	9.0	4.50	
A. Vinelandii, n. var?.	8.0	11.2	5.60	5.80
A. Vinelandii, n. var?.	8.6	12.0	6.00	
A. Vinelandii, var.....	12.2	17.1	8.55	8.57
A. Vinelandii, var.....	12.3	17.2	8.60	
"B. 29 ?".....	8.6	12.0	6.00	6.00
"B 29 ?".....	Lost	....	....	....

SOIL CULTURES

Organism	c.c. N/10 HCl used per 20 gm. soil	N. found mg.	N. found, blank subtracted. mg.	N. fixed per gm. mannite. mg.
A. chroococcum, var...	27.2	38.1	2.0	5.00
A. Vinelandii, var.....	27.2	38.1	2.0	5.00
A. Vinelandii, n. var?.	[1]28.4	39.8	3.7	8.75
"B. 29 ?".....	26.9	37.7	1.6	4.00
Uninoculated soil	25.8	36.1	...	....

[1] Some mold growth was noticed in this culture.

Remembering that the Hawaiian Islands are over 2000 miles from the nearest land area of any extent, it might, at first thought, seem hard to account for the prevalence of Azotobacter as noted in the work above reported. There are several ways, however, whereby to account for their possible introduction and distribution here. Hawaii is a land of heavy fertilization. The forty-odd sugar plantations annually expend about

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three million dollars for mixed and single element commercial fertilizers, considerable of which comes from the Pacific coast (San Francisco chiefly). Fine soil is sometimes used as a "filler" in these "mixed goods" and, as this is never sterilized, it is conceivable that it might carry Azotobacter species. Dried blood, guano and tankage are also shipped here in considerable quantity, which may also be contaminated with these microörganisms. During recent years, green manuring with legumes has been quite extensively practiced on several of the larger plantations. These legume seeds have been imported from various parts of the world and might occasionally harbor Azotobacter. Be that as it may, we are sure of one fact: Azotobacter are quite universally distributed in Hawaiian soils.

Summary

1. Thirty soils from different localities on the four large islands of the Hawaiian Group were examined as to their Azotobacter content. Their abilities to fix nitrogen in mannite solutions are given.

2. Only five soils were noted which failed to show Azotobacter growth in solutions.

3. Four different forms of Azotobacter were isolated in pure cultures, described, and their abilities to fix nitrogen in solutions and in soils given.

4. A possible explanation of the introduction and distribution of Azotobacter in these isolated island soils is advanced.

Literature Cited

(1) Lipman, C. B., and Burgess, P. S.
1915. Studies on nitrogen fixation and Azotobacter forms in soils of foreign countries. *In* Centbl. Bakt. (etc.), Abt. 2, Bd. 44, p. 481-511.

(2) Lipman, C. B., and Pressey, H. F.
1913. A contribution to our methods of determining nitrogen in humus. *In* Jour. Indus. Engin. Chem., v. 5, p. 143-144.

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THE EFFECT OF TIME AND DEPTH OF CULTIVATING A WHEAT SEED-BED UPON BACTERIAL ACTIVITY IN THE SOIL[1]

By

P. L. GAINEY, *Soil Bacteriologist, Kansas Agricultural Experiment Station*

Before the American Society of Agronomy 1914, L. E. Call (1) of the Kansas Agricultural Experiment Station, presented a very interesting paper concerning the effects that different methods of preparing a seed-bed for wheat have upon yield, soil moisture, and nitrates.

The principal fact with which we are concerned, brought out in this paper, is the very marked effects that the various experimental methods have upon the accumulation of nitrates. So striking and consistent have the results been for the past several years that a careful study of the problem was deemed advisable. A study of the bacteriological factors involved was given the writer.

The project, as conducted by the Department of Agronomy, consists in comparing eleven different methods of preparing seed beds. A detailed description of the various methods, with results secured, etc., is given in the above mentioned paper. The major points of comparison have been time of preparation and the depth of culture. The dates of preparation vary from July 15, to seeding time, about October 1. The depth of culture varies from discing to plowing 7 inches deep. In general, the effect upon nitrate accumulation has been that early and deep preparations give materially higher nitrate contents at seeding time than do late and shallow. A few specific examples from Call's paper will suffice to show the influence of these two factors.

Plot No. 1	disced	Oct. 1,	22.43 lbs.	NO_3 per acre.
Plot No. 14	plowed 3 in. deep	Sept. 15,	57.30 lbs.	NO_3 per acre
Plot No. 15	plowed 3 in. deep	July 15,	517.01 lbs.	NO_3 per acre.
Plot No. 13	plowed 7 in. deep	Sept. 15,	76.83 lbs.	NO_3 per acre.
Plot No. 10	plowed 7 in deep	Aug. 15,	255.76 lbs.	NO_3 per acre.
Plot No. 9	plowed 7 in. deep	July 15,	407.94 lbs.	NO_3 per acre.

[1] Contribution from the Research Laboratory in Soil Biology, Kansas Agricultural Experiment Station.

An abstract of this paper was read before the Society of American Bacteriologists, 1915 meeting. Received for publication May 22, 1916.

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From these data it is evident that some very important factors must be influencing the nitrate accumulation in the various plots.

For the bacteriological investigations it was deemed advisable to restrict the studies to a very limited number of plots. If the factors operating in the plots studied could be ascertained an application of similar methods to the whole series would be undertaken, provided such seemed advisable.

As representative of the extremes, both in methods of preparation and in results secured, so far as yield and nitrates are concerned, we chose as plots upon which to work Nos. 1 and 9. The treatment of No. 1 has been thorough discing at seeding time (about Oct. 1). The treatment of No. 9 has been plowing 7 inches deep July 15, and cultivating as often thereafter as necessary to insure a good seed-bed. For the four seasons '11, '12, '13 and '15, No. 1 has produced an average of 6.9 bushels of wheat per acre, while No. 9 has produced 27.59 bushels. The nitrate content for the four preceding seasons at the last analysis was for No. 1, 42.95 pounds of NO_3 per acre, and for No. 9, 318.69 pounds. From these data it is evident either that there has been much more NO_3 formed in No. 9, or that much has disappeared from No. 1. The only evident source of loss existing in No. 1 and not present in No. 9, is in the growth of weeds taking place prior to cultivation. Analysis has shown that in extreme cases this can account for only a small portion of the observed differences. It would seem then that those plots showing high NO_3 content actually form larger quantities. We have, therefore, for two seasons been directing our attention toward the nitrate forming power of soil from the two plots under study.

Experimental Data

The experiments given below include only a very few of those that have been carried out. An effort has been made to select representative experiments. At a later date a more complete report will be published.

Ammonia Forming Power

The relative ability of soil, from the two plots under study, to liberate ammonia from organic nitrogenous compounds is shown in Table I. Soil for the experiments here given was collected by means of a 2-inch soil auger. Twelve cores to a depth of 12 inches were drawn from each plot. Care was used to prevent, as far as possible, outside contamination.

The soil was brought to the laboratory, passed through a 3-mm. sieve, and thoroughly mixed. The moisture content and water holding capacity were determined. Four samples, the equivalent of 100 gm. dry soil, were then weighed. To two each of these cottonseed meal and dried-blood containing 60 mg. nitrogen were added, thoroughly mixed, and the whole placed in a 500-c.c. wide mouth bottle. The water content was made up

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to optimum (2/3 saturation), the bottle plugged loosely with cotton, and incubated at room temperature 7 days. The ammonia was determined by direct distillation in presence of magnesium oxide. If the ammonia recovered by such means is any indication of the relative ammonifying power of the respective floras, it is evident that the difference in nitrate accumulation cannot be ascribed to a difference in this phenomenon. Further, repeated measurements of the ammonia content of the soils as they came from the field, revealed no appreciable difference. This was true whether such determinations were made by direct distillation or by the aeration method of Potter and Snyder (4).

TABLE I

THE PRODUCTION OF AMMONIA IN SOIL FROM PLOTS NO. 1 AND NO. 9: MG. NITROGEN RECOVERED AS AMMONIA PER 100 GM. OF SOIL AFTER ONE WEEK INCUBATION

Date	60 mg. Nitrogen as C. S. M.				60 mg. Nitrogen as D. B.	
	Soil Plot No. 1		Soil Plot No. 9		Soil Plot No. 1	Soil Plot No. 9
Oct. 23, '14.....	26.43 mg.		29.01 mg.		15.30 mg.	14.76 mg.
Nov. 7, '14.....	28.07 mg.		30.69 mg.		7.14 mg.	6.36 mg.
	A	B	A	B		
Nov. 21, '14.....	26.15 mg.	29.82 mg	26.82 mg.	30.06 mg.		

C. S. M.—Cottonseed Meal. D. B.—Dried Blood.
A.—Surface three inches. B.—5th to 7th inches inclusive.

Nitrate Forming Power

Experiments similar to those described above have been conducted to ascertain the nitrate forming power. Table II contains a description of treatment, etc., with the averages of three such determinations. The soil for these experiments was collected October 7, October 23, and November 7, 1914. Samples were incubated for four weeks, the water lost by evaporation being replaced from time to time. Nitrates were then determined by the phenol-di-sulphonic acid method as modified by Lipman and Sharp (2).

The results here presented indicate that in the absence of any addition of nitrogen, either with or without an addition of calcium carbonate, Plot No. 1 exhibits a materially higher nitrate forming power. On the other hand plot No. 9 exhibits a materially higher nitrate forming power when nitrogen as ammonium sulphate was added with calcium carbonate, and when nitrogen in form of cottonseed meal and dried blood was added in absence of calcium carbonate. In all other instances the differences are insignificant. A careful study of the data from which this table was constructed reveals, however, consistent differences in only the first three instances mentioned, namely, when no nitrogen was added either in the

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presence or absence of calcium carbonate, and when ammonium sulphate was added in the presence of calcium carbonate. In all other comparisons sometimes Plot No. 1 and sometimes Plot No. 9 gave higher results.

The data contained in Table II indicate very strongly that, if the nitrate nitrogen produced under the experimental conditions here used gives any measure of the relative nitrate producing power, Plot No. 1 certainly contains as active a flora as No. 9. It is only just to add that in a number of experiments carried out in a similar manner, except that the nitrate content was measured at varying intervals, Plot No. 9 gave usually a somewhat more rapid accumulation during the early stages of incubation. However, such large quantities were in all cases formed in soil from Plot No. 1, as to show conclusively that the low accumulation under field conditions could not be attributed to a potentially weak flora. In addition there is absolutely no evidence to indicate a less active flora in the soil of Plot No. 1 when no nitrogen was added. Further, as pointed out above we have never been able to detect, either under field or laboratory conditions, a greater accumulation of ammonia in the soil of Plot No. 1 than in that of Plot No. 9.

TABLE II

THE PRODUCTION OF NITRATES IN SOIL FROM PLOTS NO. 1 AND NO. 9, INCUBATION FOUR WEEKS: AVERAGES OF DETERMINATIONS MADE ON DUPLICATE SAMPLES OCT. 7, OCT. 23, AND NOV. 7, 1914

Treatment		Mg. NO_3 per 100 gm. Soil	
60 mg. Nitrogen as	$CaCO_3$ gm.	Soil Plot No. 1 mg.	Soil Plot No. 9 mg.
0	0	2.93	2.26
0	1	8.43	6.85
Cottonseed Meal	0	67.02	76.59
Cottonseed Meal	1	153.89	154.45
Dried Blood	0	29.96	36.36
Dried Blood	1	102.33	103.31
Ammonium Sulphate	0	21.11	21.83
Ammonium Sulphate	1	155.23	190.51

The nitrate forming power in solution, as so vigorously recommended by Löhnis and Green (3), has also been tested. Data secured according to this method, for Sept. 14, 1915, are given below. The figures represent milligrams nitrogen converted into NO_3.

Incubation Period	10 days	28 days
Inoculum Soil of Plot No. 1	0.95 mg.	8.41 mg.
Inoculum Soil of Plot No. 9	1.36 mg.	8.47 mg.

Soil from Plot No. 1 has also been inoculated with soil from Plot No. 9 to see if the introduction of organisms from No. 9 would have any influence on the accumulation of nitrates. The results secured Sept. 14, 1915 are given in Table III. In both these last two mentioned experiments additional evidence is furnished to show that there is but slight

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difference in the nitrifying floras of the two plots under study. Similar studies, giving similar results, have been made on other plots in the series.

TABLE III

THE EFFECT OF INOCULATING SOIL FROM PLOT NO. 1 WITH SOIL FROM PLOT NO. 9: MG. NO_3 RECOVERED PER 100 GM. SOIL

Soil	Inoculum	60 mg. Nitrogen as	$CaCO_3$ gm.	After 4 weeks mg.	After 10 weeks mg.
1	0	0	0	3.32	8.20
1	1	0	0	4.00	7.50
1	9	0	0	4.00	8.20
1	0	$(NH_4)_2SO_4$	1	.....	225.00
1	1	$(NH_4)_2SO_4$	1	133.30	200.00
1	9	$(NH_4)_2SO_4$	1	150.00	200.00

While no differences in the activity of the organisms could be detected in the above experiments, it was thought that possibly by sampling the soil at different depths and making similar studies, differences might be noticed. Accordingly, samples have been taken at varying depths down to 12 inches. Under our conditions very small quantities of nitrates are formed at lower depths. The results of one such analysis are given in Table IV. Soil for these experiments was collected August 20, 1915. A

TABLE IV

SHOWING NITRATE FORMATION IN SOIL FROM DIFFERENT DEPTHS OF PLOTS NO. 1 AND NO 9: Mg. NO_3 RECOVERED PER 100 GM. SOIL

Weeks Incubated	Treatment		Soil Plot No. 1				Soil Plot No. 9			
	60 mg. Nitrogen as	$CaCO_3$ gm.	A	B	C	D	A	B	C	D
0	0	1	1.42	1.24	1.24	1.24	2.80	2.34	2.40	1.60
2	0	1	5.40	3.00	2.25	1.50	9.00	9.60	7.80	2.49
4	0	1	18.00	2.64	2.49	1.29	13.80	8.59	9.60	4.20
8	0	1	30.00	13.86	5.28	3.00	11.40	13.80	15.00	6.42
16	0	1	40.00	19.40	15.00	9.00	16.30	20.00	20.00	7.70
1	Cottonseed Meal	1	4.00	2.00	1.60	1.00	2.73	2.73	2.10	0.84
2	Cottonseed Meal	1	19.50	8.16	4.17	1.50	16.00	18.50	13.20	3.60
4	Cottonseed Meal	1	128.40	114.00	120.00	114.00	116.40	112.80	118.00	118.00
8	Cottonseed Meal	1	138.00	120.00	120.00	128.40	120.00	123.60	128.40	123.40
1	Ammonium Sulphate	1	6.40	2.66	2.00	1.42	7.20	6.90	5.20	2.00
2	Ammonium Sulphate	1	15.30	7.20	4.50	2.82	15.80	18.70	14.00	5.60
4	Ammonium Sulphate	1	189.60	114.00	133.30	60.00	180.00	180.00	180.00	75.00
8	Ammonium Sulphate	1	200.00	180.00	180.00	180.00	180.00	200.00	200.00	180.00

cylinder 5 inches in diameter was driven into the soil 12 inches deep, then was dug out and the core of soil divided into four equal parts. Five such cores were taken from each plot and the corresponding sections thoroughly mixed. Otherwise, the experiments were carried out as described earlier in the paper.

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Several very interesting facts are to be noted in the data contained in Table IV. One of the most striking is the very rapid accumulation of nitrates in the surface soil of Plot No. 1, when no nitrogen was added. Below the surface section the accumulation fell off rather rapidly. On the other hand, the second and third layers of Plot No. 9 show just as rapid accumulation as the surface layer. Not until the fourth layer is reached do we note a decrease in nitrate accumulation. The results of this particular experiment are not nearly so marked in this respect as many others that have been carried out. It is reported only because it covers a wider range than the other experiments covering the same field. The results of a similar experiment, which was continued for a year, are graphically shown in figure 1. Soil for this experiment was collected Nov. 21, 1914. Here the second layers of soil differ from those in the experiment reported above in that they include the soil from the fifth to the seventh inches inclusive, instead of that from the fourth to the sixth. We shall take up a discussion of this particular phenomenon later.

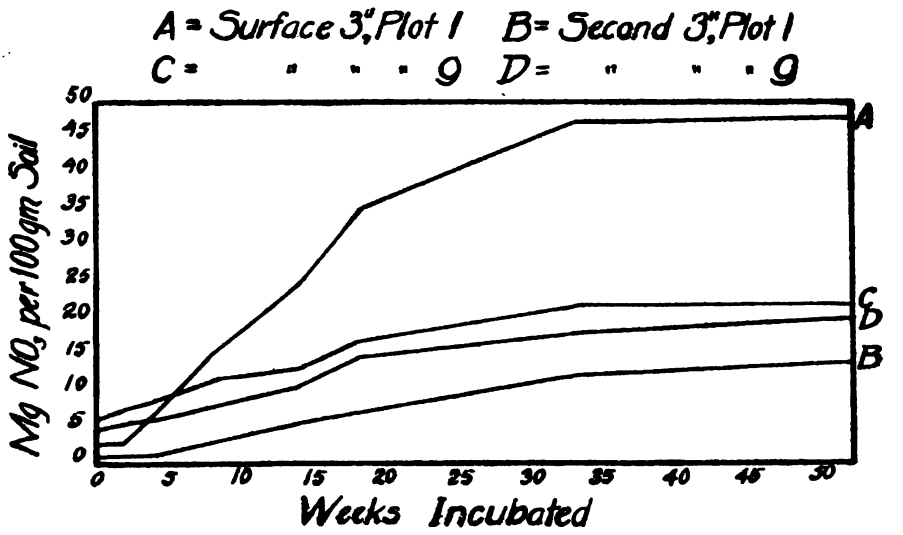

Fig. 1.—Diagram showing NO_3 accumulation in soil from different depths.

Returning to Table IV, we note that where nitrogen was added, somewhat similar results were obtained after one and after two weeks' incubation. That is, the NO_3 accumulation fell off rather rapidly immediately below the stirred area. Such differences, however, were in almost all instances eliminated by a longer incubation period. The question naturally arises as to whether this difference is due to some physical, chemical, or biological difference brought about by cultivation. We are at present in-

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terested only in biological factors. If the differences are due to variations in the nitrifying floras, there are two ways in which we might detect the same. Either by introducing the respective floras into a standard solution and measuring their activity, or by cross inoculating experiments. Experiments have been carried out by both methods and yielded alike negative results, so far as exhibiting differences between the two plots is concerned.

In solution the following quantities of nitrogen, expressed as milligrams converted into NO_3 during four weeks' incubation, were found:

	Plot No. 1	Plot No. 9
1st 3 inches	3.69 mg.	4.42 mg.
2nd 3 inches	3.07 mg.	3.30 mg.
3rd 3 inches	2.31 mg.	2.29 mg.
4th 3 inches	4.24 mg.	4.11 mg.
Average	3.33 mg.	3.53 mg.

These results, together with others secured under similar conditions, give no indication either of a material difference in the nitrifying floras of the different plots, or of the different layers of an individual plot.

An extensive series of cross inoculation experiments was not conducted at this particular date. The results reported below were secured from soil collected May 3, 1915. Duplicate samples from each layer of both plots were inoculated from every other layer of both plots, giving a total of 72 samples. Half the samples were incubated 4 weeks, the other half, 12 weeks. Only the average nitrates, expressed in milligrams NO_3 recovered from all samples inoculated from the same source, are given.

	Plot No. 1			Plot No. 9		
Inoculum	A	B	C	A	B	C
Mg. NO_3	10.3	10.3	9.9	10.4	10.4	10.4

(A—1 to 3 inches inclusive; B—5 to 7 inches inclusive; C—8 to 12 inches inclusive). When only one experiment is considered, the differences here shown are well within the experimental error.

We have, therefore, been unable to detect any consistent differences in the nitrifying power of the respective floras which could, in our opinion, explain the large differences observed in nitrate accumulation under field conditions. This being true, the controlling factors must be other than biological. We have undertaken some experimental work studying the physical and chemical factors that might be influencing the activity of the nitrifying organisms. Work along these lines is by no means complete; still, certain facts brought out may throw some light on the general problem.

From the evidence in hand, and in part presented above, two points of importance seem to be clearly demonstrated. First, transferring the soil from field to experimental conditions eliminates the differences normally

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existant. Second, the surface soil of Plot No. 1 possesses the ability to transform into nitrate nitrogen much more of its native nitrogen than does any other layer of either plot, or at least at a much more rapid rate. As accurately as we, by our present methods, are able to detect, these facts cannot be explained solely upon a difference in the respective floras either under their natural or under laboratory conditions.

The most evident changes brought about in transferring the soil from field to experimental conditions are: stirring, aerating, changing the temperature, and changing the moisture content. It is a common observation that stirring soils, apparently aside from all other influences, materially accelerates bacterial activity, and no doubt, has an influence on the phenomon under consideration. If this were the major factor, however, those layers of soil normally remaining unstirred i.e.—4 to 12 inches inclusive of No. 1, and 8 to 12 inches inclusive of No. 9, should show a greater response under the new environment. The evidence (see Table IV) does not bear this out. What has been said with regard to stirring also applies to aerating. It is hardly conceivable, though, that the surface soil of Plot No. 1 lacks aeration, yet this is the soil which gives the greatest response under laboratory conditions. In fact, Table IV shows that in all cases those soils receiving better aeration under field conditions give higher nitrate formation under laboratory conditions.

We believe temperature changes are a negative factor, since under field conditions the differences could be only very slight and in laboratory all were subjected to the same temperature.

As stated above, the soils in all experiments herein reported were made up to optimum moisture conditions, i.e. two-thirds saturated. In this respect they were materially changed from field conditions. Figure 2 illustrates the effect upon nitrate accumulation produced by varying the moisture content of soil from the experimental field. From this it can be seen that an increase of 1 per cent moisture, at or near the minimum for nitrification, may cause an increase of 100 per cent in nitrate production. If moisture is an influencing factor, however, it necessarily implies a variation of moisture content under field conditions. We have collected but little data on the moisture content of the soils under field conditions, but, through the courtesy of the Department of Agronomy, the data collected by them during the past seven years have been made available for study. They have sampled the various plots monthly in foot sections and made moisture determinations. Since the variations in nitrate content are largely brought about during August and September and almost entirely in the first foot of soil, we have confined our studies to the data collected during those two months, and for the first foot sections. It could not be expected that such meagre data would give us a clear insight into the relation existing between moisture content and nitrate pro-

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duction. However, a remarkably close agreement can be traced for some seasons. Figure 3 illustrates graphically the correlation between the two factors for the season 1911. Similar relations, though not so close, can be traced for all other years for which data are available, except 1913.

It will be remembered that 1913 was omitted from the averages of yields and nitrates present in plots No. 1 and No. 9 given earlier in the paper. This was done because of the uniform results secured for that season. The yield was on Plot No. 1, 21.83 bushels, and on No. 9, 25.83 bushels of wheat per acre, a difference of only 4 bushels, while checks showed showed a variation of 6.25 bushels. The difference in nitrate content was at seeding time only 3 pounds per acre in favor of No. 9. The greatest variation in NO_3 content in the whole series was between

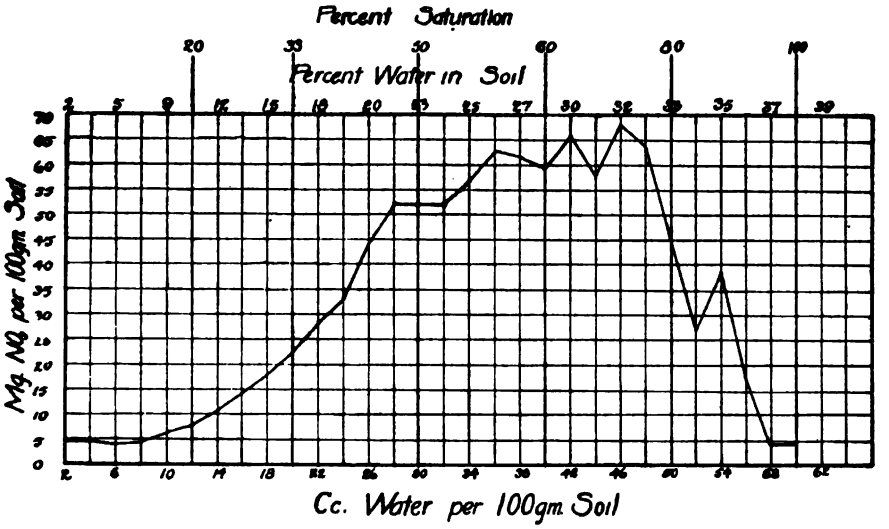

Fig. 2.—Diagram showing the effect of varying water content upon NO_3 formation in soil from experiment field.

checks. The same is true regarding moisture content. In other words, the whole series gave negative results for this season. A glance at figure 4, illustrating graphically moisture and nitrate content for 1913, shows absolutely no correlation between moisture content during August and September and nitrate content. In figures 3 and 4 the moisture lines give the average per cent of moisture actually available for nitrifying organisms when the August and September analyses were made. The nitrate lines represent pounds of NO_3 per acre three feet at the October analysis. We do not wish to be understood as saying that the large differences observed under field conditions can be entireely explained upon a moisture content basis. We do believe, however, that the few facts presented above are of considerable significance.

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Turning our attention for a moment to the differences exhibited under laboratory conditions between the various layers of soil as illustrated in figure 1, it may be asked, how can these be explained? No evidence indicating a lack of active organisms in the lower layer soil of Plot No. 1 has been secured. The differences then must be due either to an inhibiting agent in the lower layer or to a difference, either in quantity or quality, of nitrogen present in the two layers. The fact that added nitrogen in any readily available form has been rapidly nitrified in the lower layer would seem to preclude the first possibility. As to the second possibility, it was found that the surface layer contained approximately 100 parts per million, or actually 100 pounds per acre, more nitrogen than the sec-

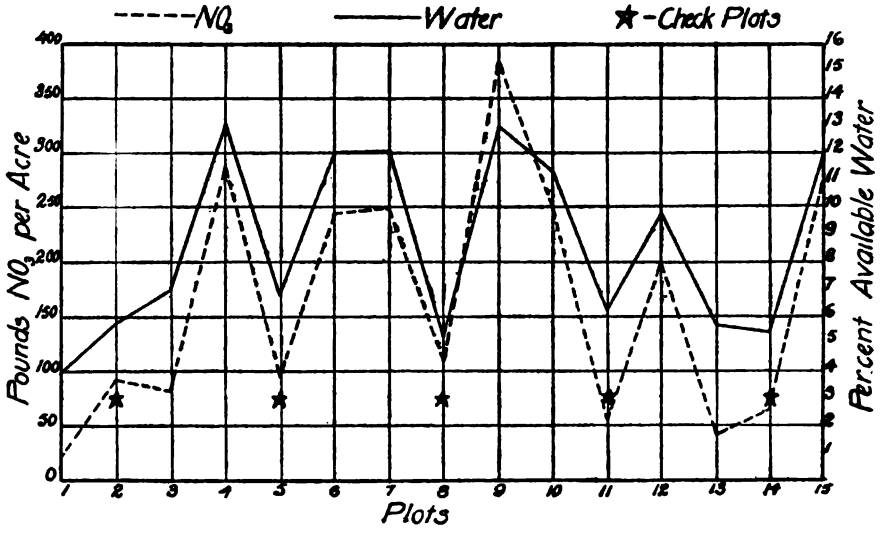

Fig. 3.—Diagram showing the relation between available water and NO_3 content under field conditions, 1911.

ond. There are, however, approximately 1500 parts per million nitrogen in the second layer, and unless this 100 parts per million exists in a different form or condition it would appear relatively insignificant. Besides, the second layer soil of Plot No. 1 contained slightly more nitrogen than either layer of Plot No. 9, yet, it failed to produce as much nitrates. As pointed out earlier, no difference could be detected in the ammonia content. This is rather significant since ammonia is the immediate forerunner of nitrates. Observations indicated a much larger quantity of undecomposed organic matter in surface soil from Plot No. 1 than in any of the other layers under study. By an arbitrary specific gravity method of separating this organic matter it was found in the following quantities in the four soils:

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Surface layer No. 1 13,500 parts per million
Surface layer No. 9 3,400 parts per million
Second layer No. 1 2,200 parts per million
Second layer No. 9 3,700 parts per million

The nitrogen contents of this organic matter were in practically the same proportions. The excess of undecomposed organic matter in the surface layer of Plot No. 1, therefore, contained approximately 100 pounds of nitrogen, a figure just equal to the difference in total nitrogen between the two layers. A glance at figure 2 will show that when the NO_3 content of the surface soil of Plot No. 1 exceeds the second layer by approximately 100 pounds of nitrogen, the production in the two layers runs almost parallel (32nd to 52nd week). In other words, it appears

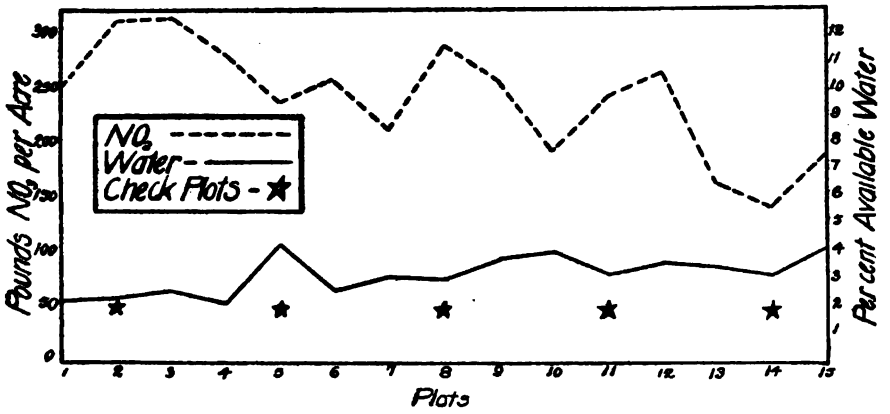

Fig. 4.—Diagram showing the relation between available water and NO_3 content under field conditions, 1913.

that something has prevented the decomposition of organic matter in the surface soil of Plot No. 1 under field conditions. This is, no doubt, due to the fact that the cultural method followed on this plot, i. e. discing, leaves the organic matter on or near the surface where, because of the normally dry conditions during the summer and fall months, decomposition is impossible. On the other hand, deep plowing incorporates the organic matter throughout the first 7 inches. More moisture being present, both because of depth and early culture, decomposition is made possible.

Summary

As a summary we may state that the evidence here submitted indicates that:

1. The differences in nitrate content reported by Call cannot be attributed to a difference in the bacterial content.

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2. Some non-biological condition existing in certain plots, under field conditions, prevents the normal activity of the bacterial flora.

3. Among the factors controlling bacterial activity the available moisture probably plays a paramount rôle.

ACKNOWLEDGEMENT

The writer wishes to express his appreciation of the valuable help rendered by the Department of Agronomy, through Prof. L. E. Call, in furnishing such data as have been used relative to yields, and moisture and nitrate content under field conditions.

LITERATURE CITED

(1) CALL, L. E.
1915. The effect of different methods of preparing a seed-bed for winter wheat upon yield, soil moisture and nitrates. *In* Jour. Amer. Soc. Agron., v. 6, p. 149-169.

(2) LIPMAN, C. B., and SHARP, L. T.
1912. Studies on the phenol-disulphoric acid method for the determination of nitrates in soils. *In* Univ. Cal. Pub. Agr. Sci., v. 1, p. 21-37.

(3) LÖHNIS, F., and GREEN, H. H.
1914. Methods in soil bacteriology: vii. Ammonification and nitrification in soils and solutions. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 40, p. 457-479.

(4) POTTER, R. S., and SNYDER, R. S.
1914. The determination of ammonia in soils. Iowa Research Bul. 17, p. 1-19.

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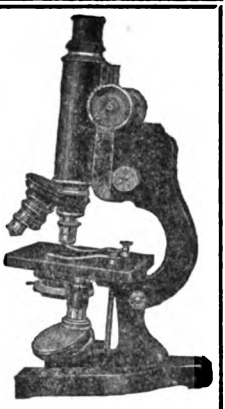

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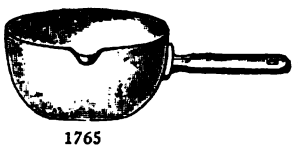

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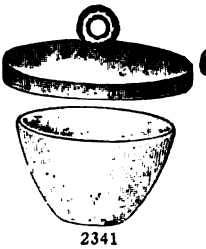

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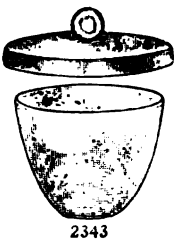

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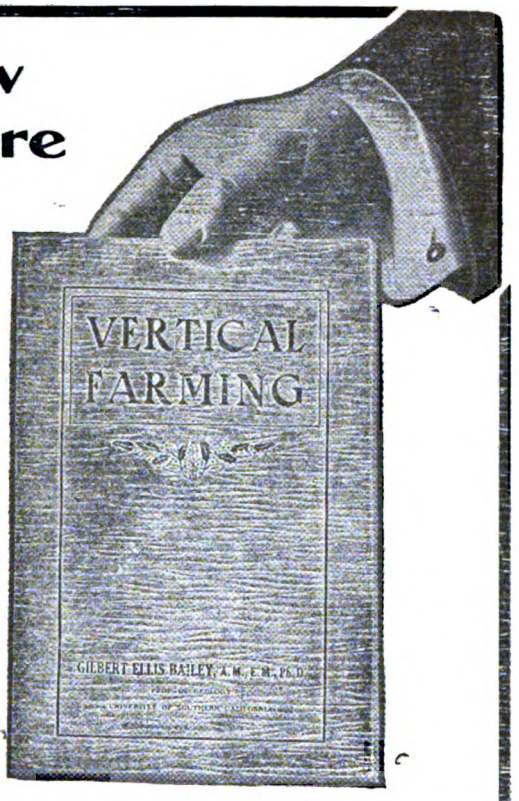

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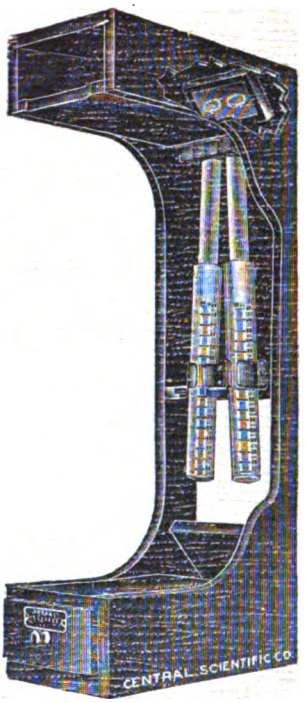

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VOL. II SEPTEMBER, 1916 No. 3

SOIL SCIENCE

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SOIL SCIENCE

A · MONTHLY · JOURNAL · DEVOTED TO · PROBLEMS · IN · SOIL · PHYSICS SOIL · CHEMISTRY · AND · SOIL · BIOLOGY

Volume II Number 3

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SOIL SCIENCE

RUTGERS COLLEGE

VOL. II NEW BRUNSWICK, N. J., SEPTEMBER, 1916 No. 3

SULFUR ON ALKALI SOILS

European and American investigators have shown convincingly that elementary sulfur may be readily oxidized in the soil by microörganisms. Pure cultures of these microörganisms have not yet been isolated. Preliminary work done in the laboratories of the New Jersey Agricultural Experiment Station shows that the oxidation of elementary sulfur may be accomplished by certain types of bacteria, and possibly also by molds. There is reason to believe that inoculation with pure cultures of sulfofying bacteria may find a place in progressive methods of soil treatment.

Meanwhile, it is quite certain that elementary sulfur, used in small or large amounts may be transformed by microörganisms into sulfuric acid. It is not proposed to discuss at this time the value of sulfuric acid thus formed for modifying the soil reaction. It need only be said in passing that the application of elementary sulfur has been found to be of great service in checking the development of scab on potato tubers. In a recent contribution to SOIL SCIENCE from the New Jersey Station, it was also shown that sulfur oxidation in soil may be made to play an important part in the utilization of insoluble phosphates by crops. Work now in progress has already disclosed a number of problems of great scientific and practical significance.

The investigations of C. B. Lipman, of the University of California, on the value of sulfuric acid in the reclamation of certain alkali soils would justify the suggestion that elementary sulfur could be used to great advantage instead of sulfuric acid as proposed. As is well known, there are vast areas of land in which carbonates and bicarbonates of sodium are present in sufficient amount to interfere partially or completely with profitable crop production. It is hoped, therefore, that experiments will be undertaken with sulfur as a means of reclaiming land which now contains an excess of these carbonates and bicarbonates.

JACOB G. LIPMAN.

August 24, 1916.

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PHYSIOLOGICAL BALANCE OF NUTRIENT SOLUTIONS FOR PLANTS IN SAND CULTURES[1]

By

ARTUHR G. MCCALL, *in Charge of Soil Investigations, Maryland Agricultural Experiment Station*

ABSTRACT

The experiments described in this paper were conducted for the purpose of studying the relative growth rates of young winter wheat seedlings, when grown in a substratum of sand and supplied with a nutrient solution of the same initial total concentration, but varying in the proportions of the component salts. An initial total concentration of 1.75 atmospheres maximum osmotic pressure was employed for 36 different proportions of the three component salts, KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$.

The series included all of the possible proportions of the three salts, when the components are made to vary by increments of one-tenth of the total possible osmotic pressure. Each culture consisted of 6 plants growing in washed quartz sand. The enameled steel pots employed were approximately 12 x 12 cm., inside diameter, and held 1500 gm. of dry sand. After the seedlings were planted the surface of the sand was covered with a thin layer of wax to prevent loss of moisture by evaporation. The solutions were renewed every three days by the addition of 250 c.c. of fresh solution through a funnel which occupied a position at the center of the pot. At the same time that the fresh solution was being added at the top, the old solution was removed from the bottom of the pot by means of suction applied to a small tube connecting with the interior. The total growth period was 24 days, during which time the total water loss from each culture was determined at the end of each 3-day interval. At the end of the growth period the cultures were compared with respect to: (1) dry weight of tops, (2) dry weight of roots, (3) total water loss, (4) water requirement per gram of dry tops, (5) water requirement per gram of dry roots, and (6) the ratio of the weight of tops to dry weight of roots.

Three preliminary series of wheat cultures were grown in sand and supplied with nutrient solutions having a range in initial total concentration from 0.2 to 5.0 atmospheres. One of these series was characterized by having 5 tenths of the total osmotic concentration derived from monopotassium phosphate, 2 tenths from calcium nitrate, and 3 tenths from

[1] Botanical contribution from the Johns Hopkins University, No. 52.

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magnesium sulphate, while the other two had 4 tenths of the total osmotic concentration derived from KH_2PO_4, 3 tenths from $Ca(NO_3)_2$, and 3 tenths from $MgSO_4$. These three series were in accord in showing that a total concentration of 1.75 atmospheres was well within the range required for optimal growth of wheat tops.

The main results may be summarized as follows:

1. The graphs representing the growth rate of young wheat plants, for three preliminary series, show a region of optimal growth rate lying between the concentrations 1.0 and 2.0 atmospheres.

2. With the initial total concentration about 1.75 atmospheres, the nutrient solution that produced the greatest dry weight of tops also produced the greatest dry weight of roots. This solution is characterized by having 2 tenths of the total osmotic concentration derived from KH_2PO_4, 7 tenths from $Ca(NO_3)_2$, and 1 tenth from $MgSO_4$.

3. A general comparison of the results from this sand culture series with solution cultures (Shive's) grown from the same lot of seed but at a different time period brings out some interesting comparisons, which may be summarized as follows: (1) the average dry weights of both tops and roots were decidedly greater for the plants grown in the sand than for those grown in the solutions, (2) the results obtained in the solution culture series having a total osmotic concentration of 0.1 atmosphere, are more nearly like those from the sand series than are the results secured from the more concentrated solution series (1.75 atm.) in which the solutions were of the same total osmotic concentration as that employed for the sand cultures, and (3) there is a marked difference between the solutions producing the best development of plants in sand and those giving the best growth in the solution cultures, with respect to the osmotic proportions of the three salts employed.

4. A comparison of the results from these two series, the one grown in solution and the other in sand cultures, furnishes evidence for the conclusion that selective adsorption plays an important rôle in bringing about the observed physiological differences.

5. The sand culture solutions giving *low* yields of tops are characterized by a *wide* range in the Mg/Ca ratio; a very wide range in the Mg/K ratio, and a narrow range in the Ca/K ratio value. The solutions giving *high* yields of tops show a *narrow* range in the Mg/Ca ratio; and a comparatively wide range in both the Mg/K and Ca/K ratio values.

6. The data presented support the conclusion of earlier workers to the effect that the total transpirational loss from a plant culture is approximately proportional to the growth made by the plants during the period of time considered.

7. The water requirement per gram of dry tops varies considerably with the different proportions of the component salts. From these data it appears that *low* water requirement for tops is associated with a *low*

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partial osmotic concentration of mono-potassium phosphate, and that *high* water requirement is associated with *high* partial concentrations of both magnesium sulphate and mono-potassium phosphate.

8. The water requirement per gram of dry roots is much higher than the same value for tops.

9. A consideration of the ratio of tops to roots brings out the fact that in every instance a high water requirement corresponds to a high ratio of tops to roots.

10. Good growth of tops was found to be associated with a high osmotic ratio of $Ca(NO_3)_2$ to $MgSO_4$ and poor growth of tops with a low value of this ratio. We are not justified, however, in drawing, from these results, any definite conclusions with respect to the calcium-magnesium ratio as such, since much of the superior growth, in the cultures where $Ca(NO_3)_2$ was in excess, may be ascribed to the presence of a large amount of the NO_3 radical, which is known to be favorable to very vigorous vegetative growth.

Introduction

During the closing years of the seventeenth century Woodward (46) grew spearmint, potatoes and vetch in rain, spring, river, conduit and distilled water, for the purpose of determining whether it was the water or the solid soil particles which nourished plants. It appears, however, that water cultures were not employed for the purpose of studying plant nutrition to any great extent until about 1859, at which time Knop (16) and Sachs (32) began their investigations of Liebig's theory that the materials dissolved in the soil water are not generally sufficient for plant growth and that plants derive food directly from the soil particles.

This early work of Knop and of Sachs, together with their subsequent experiments along the same lines, gave such an impetus to water-culture work that there has grown up, during the past half century, a very extensive literature upon the subject of water or solution cultures. Most of the earlier publications in this field are to be found in *Die Landwirtschaftliche Versuchs-Stationen,* while a general review of the literature may be found in such works as those of Pfeffer (30), Duggar (9), and Czapek (7). The very recent work of Tottingham (40) and the publications of Shive (37, 38) have added a very important and interesting chapter to the already voluminous literature upon the subject of plant nutrition with special reference to the physiological requirements of the plant.

After an extensive chemical study of the components of a standard formula, Tottingham grew preliminary cultures of wheat in two forms of Knop's solution, one including mono-potassium phosphate and the other having the phosphate in the di-potassium form. In these cultures, the solution containing the mono-potassium phosphate produced 17.8

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per cent better growth of tops and 17.5 per cent better growth of roots than the solution containing the di-potassium salt. Following this preliminary work, Tottingham employed 84 different solutions, all of approximately the same total osmotic concentration, but each culture differing from all of the others in the proportions of the four salts, mono-potassium phosphate, potassium nitrate, calcium nitrate, and magnesium sulphate. To each of the cultures was added the usual trace of iron, in the form of ferric phosphate. With a total concentration at about the optimum for young wheat seedlings, the solution having the proper salt proportions to give the best growth of tops was found to produce an improvement of 11 per cent, based on the dry weight of tops grown in Knop's solution of the same total osmotic concentration.

Repeating some of these tests, Shive obtained results that show a very close agreement with those previously reported. His best growth was secured with the same proportions of salts as those found best by Tottingham, and an improvement of 12 per cent over Knop's solutions was obtained.

As a result of the further study of these four-salt solutions, Shive has been able to make a combination of three nutrient salts which contain all of the essential elements of plant growth (with the exception of iron) and which does not form a precipitate in solutions of the required concentration. The solutions employed by this writer contain monopotassium phosphate, calcium nitrate and magnesium sulphate, and differ from the four-salt solutions just mentioned by omission of potassium nitrate. The three salts dissociate in dilute solutions, to form all of the ions that are found in the four-salt mixture of Knop or Tottingham. Testing this three-salt solution by the same general method as was employed by Tottingham, Shive secured cultures showing an improvement of 27 per cent over Knop's solution of the same total concentration. With this series of wheat cultures was included Tottingham's best solution, which showed a corresponding increase over Knop's solution of but 16 per cent.

Upon learning of these recent results, the writer became greatly impressed with the desirability of studying the effect of these solutions upon plants grown in sand where some of the physical environmental conditions of the soil are present, but where the cultures are relatively unaffected by the biological complications introduced when ordinary soils are used. The work of Tottingham and that of Shive were confined exclusively to water cultures, in which the solution was renewed at frequent intervals. Accordingly, it was planned to repeat a part of the work of Shive, using the same three-salt solution as was employed by him and the same kind of plant (wheat), but employing pure sand as the substratum, instead of having the roots of the plants completely immersed in the free solution. In order to secure a renewal of the nutrient solu-

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tions at intervals during the growth of the plant, a special method (25) was devised, whereby the old nutrient solution could be removed and fresh solution added to the pots, without seriously disturbing the relation between the roots and the sand.

This investigation was conducted in the Laboratory of Plant Physiology of the Johns Hopkins University, under the direction of Dr. B. E. Livingston, to whom the author is deeply indebted for many criticisms and helpful suggestions.

METHODS

I. Sand Cultures with Renewed Solutions

After a great deal of preliminary work, the following method was adopted as best suited to the needs of the experiment. The pots used were of enameled steel ("graniteware"), approximately 12 x 12 cm., inside diameter, narrowing slightly toward the base and having a wide projecting rim at the top. When filled to within about 3 cm. of the top, these pots hold 1500 gm. of dry quartz sand. To provide for the removal of the solution a small lead tube is soldered into the side as near the bottom as possible.[1] The soldered joint and the lead tube are covered with paraffin, to guard against possible lead-poisoning, and the outlet of the tube closed by means of a short length of rubber tubing provided with a pinch-cock. The accompanying photograph, Plate I, shows the form of the pot and gives a good general idea of the appearance of the cultures at a period of about 20 days after planting. The description of the method given in the following paragraphs includes the details of manipulation from the starting of the seedlings to the harvesting of the plants.

The seed is soaked in water and the seedlings grown, in the manner described by Tottingham, to a height of about 3 or 4 cm., when they are ready to be transferred to the sand cultures. While the seed is being germinated, 1500 gm. of dry quartz sand (previously washed several times with distilled water) are weighed into the pot, the outlet at the bottom of the pot being screened on the inside by means of a plug of glass wool inserted before the pot is filled. With the pinch-cock closed, distilled water is now added to the pot until the sand is completely saturated, after which the pinch-cock is opened and the surplus water allowed to drain out through the tube at the bottom of the pot, until the last free water has disappeared from the surface of the sand. An inverted hemispherical porcelain funnel is placed in position at the center of the soil surface, as shown in Plate I, and the pot is then ready to receive the seedlings.

After careful selection for uniformity, the seedlings (six in number)

[1] In order to make the hole in the side of the pot it is necessary first to chip off a small piece of enamel with the sharp point of a file. This serves to give entrance to the point of a small twist drill which then passes through the iron and chips off the enamel on the inside, thus exposing sufficient iron to give adherence to the solder.

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are planted, being equally spaced on a circle lying midway between the edge of the funnel and the wall of the pot. Care is taken to place the seedlings at such depth that the top of the grain is just level with the surface of the sand. After all of the seedlings are in place, the pinch-cock is closed, and the pot is tapped gently on the table until free water appears on the surface of the sand. This manipulation serves to pack the sand around the roots of the seedlings and at the same time to level the surface of the sand preparatory to putting on the seal of Briggs and Shantz (4) wax. This wax is composed of 80 per cent paraffin and 20 per cent petrolatum, the exact proportions being unimportant. The mixture has such a low melting point and is such a poor heat conductor that it can be poured around the most delicate seedlings without injury.[1] The surplus water is then drawn out of the pot by application of suction (by means of a water-aspirator) to the tube at the bottom, and a thin layer of the melted wax is flowed over the surface, completely covering the sand between the funnel and the wall of the pot. Care should be taken to have the wax only a few degrees above its melting point or the seedlings may be injured at the point of contact with the wax. The surface must be sealed to prevent the loss of water by evaporation from the surface of the sand, and, of course, the walls of the pot must be impervious to moisture in order that transpiration can be measured and the concentration of the nutrient solution controlled.

The pot is now ready to receive the nutrient solution, which is added through the funnel at the top while the water is being removed at the bottom by the application of suction to the outlet tube. A double or triple portion of the nutrient solution is passed through the sand at this first application, in order to flush out the distilled water. The pot is now placed on the balance and the removal of solution is continued until the sand has been reduced to the desired moisture content, which should be, as nearly as possible, the optimum for plant growth. At the end of each 3-day period the pot is again weighed, and sufficient water is added through the funnel to bring the entire system back to its original weight. A fresh nutrient solution is then added (250 c.c. for pots of this size), while an equivalent quantity of solution is removed at the bottom. A nutrient solution of the same concentration may be used throughout the entire period of growth, or it may be varied from time to time as the plants continue to develop. The plants may be harvested at any time by removing the wax seal and cutting them level with the surface of the sand. If desired, the roots may be recovered from the sand by washing them out with a jet of water. The records of pot weights give the amount

[1] The writer has found the paraffin sold under the trade name "Parawax" to be cheap and very satisfactory. Care must be taken to secure a good grade of petrolatum or vaseline. Some brands seem to contain volatile substances which cause injury to the plants at the point of contact with the seal. The Chesebrough brand of white vaseline has been found to be safe to use for this purpose.

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of water lost by each culture (transpiration) and the harvest records may be made to include the dry weights of both tops and roots.

II. Materials Used

The substratum used in these cultures consisted of medium-fine white sand,[1] which had been previously washed four times with distilled water from a Barnstead still. For the first washing a 2-gallon glazed stoneware jar was filled about two-thirds full of distilled water and the dry sand slowly poured in while the contents of the jar were kept agitated by means of a large glass stirrer. The surplus water was then decanted, after which the sand was spread out on large sheets of paper until air-dry. The dry washed sand was then weighed into the granite-ware pots and was afterwards washed three times by covering with distilled water and drawing the water through the material by means of suction applied to the tube at the bottom of the pot. Failure of control cultures to develop in the sand supplied only with distilled water, instead of with the nutritive solution, gave conclusive proof that this washing treatment was sufficient to remove any nutrient salts that might have been in the unwashed sand.

The salts used in making up the culture solutions were Baker's "analyzed" mono-potassium phosphate and calcium nitrate and Merck's "blue label" magnesium sulphate. Stock solutions were prepared by dissolving gram-molecular weights of the salts separately in Jena flasks, each solution being made up to a volume of one liter. Before making up the final nutrient solutions the stock solutions were diluted to one-fourth molecular and stored in flasks, each of the latter being connected to a burette with automatic filling arrangement. By means of these burettes the required amounts of solution were drawn at each time when a new set of nutrient solutions were to be prepared. The drying of the salts, the making up of the stock solutions, and all of the other manipulations with respect to the making up of the nutritive solution were substantially the same as those described by Shive (38).

III. Culture Solutions

The growth-rate of a plant is determined by two sets of conditions, one of which is internal to the plant and hence thus far very largely beyond our control, while the other is external, or environmental, and hence subject more or less to artificial control. In the present work an attempt

[1] A mechanical separation of this sand gave the following percentages of different sized particles:

Fine Gravel (2.0 to 1.0 mm.)	Coarse Sand (1.0 to 0.5 mm.)	Medium Sand (0.5 to 0.25 mm.)	Fine Sand (0.25 to 0.1 mm.)	Very Fine Sand (0.1 to 0.05 mm.)
0.14	48.62	26.40	22.88	1.46

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was made to have the internal conditions of the various plants as nearly uniform as it was possible to make them, by starting a large number of seedlings and selecting plants of uniform size and appearance. The environmental conditions may be further divided into two groups, one of which may be defined as aerial and the other as subterranean. Since the present study concerns the subterranean environment it was essential that the aerial environment should be made as uniform as possible for all of the cultures. This uniformity in aerial conditions is best secured by placing the cultures on rotating tables, as described by Shive, thus exposing all of the plants to approximately similar changes of heat, light, and moisture conditions. However, since rotating tables were not available for this work, a less convenient method was employed: the cultures were shifted in position each day, in regular order, on a stationary table. In order to avoid unequal shading, as far as possible, the cultures were placed in two single rows extending east and west in the greenhouse, the rows being of sufficient distance apart so that at no time during the day was there any shading of one row by the plants in the other. As a further precaution, each row of pots stood on narrow slabs of slate which were elevated about 15 cm. above the general level of the table.

In studying the comparative physiological effects of different nutrient solutions in such cultures as these, it is of course desirable to have uniformity in all of the subterranean conditions affecting the plant, excepting those conditions that are dependent upon the properties of the nutrient solutions, but this is difficult of accomplishment. A fairly satisfactory degree of uniformity in the subterranean physical conditions was secured by filling all of the pots from the same bulk sample of sifted sand and by taking care to maintain the same amount of moisture in all of the pots throughout the duration of the experiment. As has been pointed out by Livingston (18) and others, the cultural solution may influence the plant in two different ways. The chemical effect of the solution is dependent upon the chemical nature of the salts present and also upon the relative amounts of the different salts contained in the nutrient solution. On the other hand, the solution may exercise a marked influence upon plant growth in a purely physical way, by virtue of its total concentration to which is related the osmotic equilibrium between the nutrient solution outside the roots and the cell sap within. When water cultures are employed, the total concentration of the solution with which the roots are in contact is known with a fair degree of accuracy, but in sand cultures the solution may undergo a change not only in its total concentration but also in the relative proportions of the different salts, as the result of its contact with the solid particles of the substratum.

Elaborate investigations concerning the relation between the concentration of the solution in the soil and the growth of plants have resulted in disappointment largely because of the fact that, while it has been admitted that the adsorbed layer at the immediate surface of the soil grain

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is of different concentration from that of the mass of free solution, it appears that no method has yet been devised for determining this difference in concentration. Furthermore, no direct experimental evidence has been reported that would throw light upon the question of the availability and the non-availability of salts in the adsorbed layer. It has been suggested that the thickness of the adsorbed layer is frequently less than that of the outer cell walls that cover the absorbing protoplasm in epidermal cells of roots. From this, it is argued that, since the protoplasm does not come into direct contact with the adsorbed layers about the soil grains, this layer, therefore, must be unavailable to the plant except for the possible slow diffusion of the salts from the adsorbed layer out into the adjacent free-water film. As a further support for the theory that the adsorbed layer is not available to plants, it is pointed out that the addition of a few pounds of a soluble potash salt to an acre foot of soil, for instance, often produces a very decided increase in the growth of the crop, although there may be present in the upper foot of soil as much as 10,000 pounds of potassium. This is interpreted to mean that the salt originally present in the soil solution was practically all in the adsorbed layers and that the relatively slight addition resulted in increasing the concentration of the free solution.

The effect of adsorption in reducing the toxicity of certain dissolved substances has been observed by several investigators.

True and Oglevee (41) studied the effect of the addition of difficultly soluble substances to toxic electrolytes and non-electrolytes. Sand, filter paper and paraffin were added to dilute solutions of the toxic substances and the changes produced by the addition of these materials were detected by observing the growth rate of the primary root of *Lupinus albus* when emersed in the different solutions for a period of 24 hours. The presence of these insoluble substances in the toxic solutions always gave an accelerated growth rate, the effect being quite similar to that produced by simple dilution. These investigators regard it as probable that the substances added to the toxic solutions acted as adsorbing surfaces for the molecules or the ions of the toxic substances dissolved in the liquid. This would affect the solution much like the addition of water, to bring about a decreased number of molecules or ions in a given volume of free solution. Similar results were obtained by Breazeale (2).

In a paper under the same title published the following year, these last mentioned authors (42) cite the work of Nägeli, with *Spirogyra* growing in distilled water obtained from copper containers. Nägeli (27) had found that his solutions, containing minute traces of a toxic metal, could be made harmless by the addition of paraffin, graphite, filter paper, or glass.

Dandeno (8) has also studied the effect of the addition of finely divided soils to toxic solutions. He found that the effect of the addition of non-chemical bodies to toxic solutions very much retarded the action

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of the toxic substances in bringing death to the radicles of plants growing in such solutions.

Jensen (15) has shown that the introduction of pure quartz flour into a toxic solution reduces its toxicity to a marked degree. He states, however, that it is an open question whether the reduction in toxicity is due (1) to adsorption, (2) to reduced freedom of movement of the solute particles (that is, a reduction of the diffusion rate), or (3) to possible chemical changes induced by the presence of the finely divided quartz.

Breazeale (1) quotes some of Livingston's unpublished data, to show that in soil or in sand cultures the effect of concentration is quite different from that found in water cultures. These data indicate that the concentration best suited to the growth of wheat in water cultures is about 300 parts per million, while in sand cultures the solution giving the best growth rate has an initial concentration of approximately 2500 parts per million.

The present investigation furnishes some very important evidence concerning the availability of the adsorbed salts, which evidence will be discussed after the experimental results have been presented.

Following the nomenclature employed by Tottingham (40) and Shive (38), the concentrations used will be expressed in terms of osmotic concentration (maximum osmotic pressure in atmospheres[1]), or in terms of gram-molecules per liter of solution.

Experimentation

I. Determination of the Optimal Total Concentration

Working with water cultures, Tottingham (40) secured his best growth of wheat seedlings in a solution having a total osmotic concentration of 2.50 atmospheres, but he calls attention to the fact that this concentration may be somewhat above that required for optimal growth, and Shive (38) has shown that a total concentration of 1.75 atmospheres of maximum osmotic pressure lies within the range of concentration required for the best growth of wheat seedlings.

To determine if these approximately optimal total concentrations, found for water cultures, would hold good for sand cultures, three preliminary series of sand cultures were grown. The cultures of each series all received solutions having the same proportions of the three component salts, but the total osmotic concentration of the solution used was different for the various individual cultures in the same series. These preliminary cultures will be designated as Series A, Series B, and Series C. Series A and B were grown simultaneously from April 9 to April 29, while Series C was started April 28 and harvested May 18. Each series consisted of 6 cultures, differing from each other, as has been mentioned, in the total concentration of the nutrient solution employed, but all cultures

[1] For a discussion of the methods used for measuring the physical properties of solutions, and an explanation of the terms employed, see Findlay, Alexander (10), Washburn, E. W. (43).

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in the same series having the same salt proportions. The solutions for Series A ranged in concentration from a minimum of 0.5 of an atmosphere to a maximum of 3.0 atmospheres, and were further characterized

TABLE I

DRY WEIGHTS OF TOPS OF WHEAT GROWN FOR 20 DAYS IN SAND, WITH THREE-SALT SOLUTIONS VARYING IN TOTAL CONCENTRATION FROM 0.5 TO 3.5 ATMOSPHERES

Series A, conducted from April 9 to April 29, 1915

Culture No.	Total Concentration of Solution	Amount of Molecular Solution Required per Liter of Nutrient Solution			Dry Weights	
		KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	Absolute	Relative to Culture 1
	atm.	c.c.	c.c.	c.c.	gm.	
1	0.5	5.1	1.5	4.3	0.8022	1.00
2	1.0	10.2	3.0	8.6	1.0498	1.31
3	1.5	15.3	4.5	12.9	1.0998	1.37
4	2.0	20.4	6.0	17.2	1.2454	1.55
5	2.5	25.5	7.5	21.5	1.0006	1.25
6	3.5	35.7	10.5	30.1	0.8678	1.08

by having 5 tenths of the total osmotic concentration derived from mono-potassium phosphate, 2 tenths from calcium nitrate, and the remaining 3 tenths from magnesium sulphate. The solutions employed in Series B

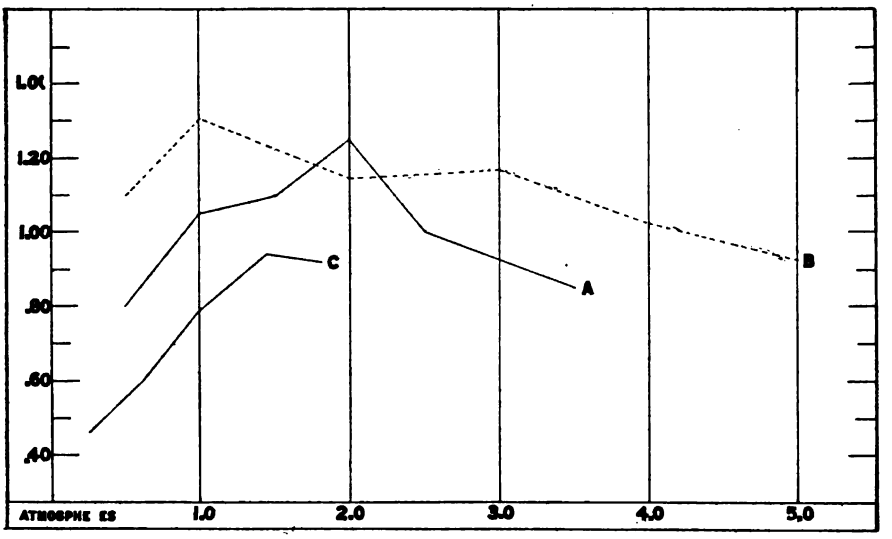

Fig. 1.—Dry weights of wheat grown for 20 days in sand cultures, with a three-salt solution varying from 0.1 atmosphere to 5.0 atmospheres total osmotic concentration.

had a range in total concentration from a minimum of 0.5 of an atmosphere to a maximum of 5.0 atmospheres, and derived 4 tenths of the total concentration from mono-potassium phosphate, 3 tenths from calcium

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nitrate, and 3 tenths from magnesium sulphate. In Series C the solution ranged in total concentration from 0.2 of an atmosphere to 1.8 atmospheres, with the relative proportions of the three salts the same as in Series B. These particular sets of salt proportions were selected for the preliminary series because of the fact that Shive had already shown that these are associated with high yields of tops in solution cultures.

The data concerning the yield of tops in these preliminary series are given in Tables I, II and III. In these tables the first column gives the culture numbers; the second column shows the total concentration of the solutions employed, stated (in terms of maximum osmotic pressure) as atmospheres or fractions of an atmosphere. The three succeeding columns show, in each instance, the volume of stock molecular solution necessary for a liter of the required nutrient solution. Then follow two columns, one of which is devoted to the absolute, and the other to the relative dry weights of tops, the latter expressed in terms of Culture 1,

TABLE II

DRY WEIGHTS OF TOPS OF WHEAT GROWN FOR 20 DAYS IN SAND, WITH THREE-SALT SOLUTIONS VARYING IN TOTAL CONCENTRATION FROM 0.5 TO 5.0 ATMOSPHERES

Series B, conducted from April 9 to April 29, 1915

No. Culture	Total Concentration of Solution	Amount of Molecular Solution Required per Liter of Nutrient Solution			Dry Weights	
		KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	Absolute	Relative to Culture 1
	atm.	c.c.	c.c.	c.c.	gm.	
1	0.5	4.6	2.5	5.3	1.1074	1.00
2	1.0	9.2	5.0	10.6	1.3347	1.21
3	2.0	18.4	10.0	21.2	1.1430	1.03
4	3.0	27.6	15.0	31.8	1.1749	1.06
5	4.0	36.8	20.0	42.4	1.0210	.92
6	5.0	46.0	25.0	53.5	0.9256	.84

taken as unity. The relative dry weights obtained in these series are shown in the graphs of figure 1. From an inspection of the graphs it will be seen that (with the osmotic proportions of the three salts here employed) the best growth of tops in sand cultures was obtained by the use of nutrient solutions with total concentration between 1 and 2 atmospheres. Since the concentration of Shive's optimal water-culture solution (1.75 atm.) is within the range of optimal concentrations as shown by these preliminary sand cultures, that concentration was employed in the subsequent work here to be reported.

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II. *Determination of the Effect of Thirty-six Different Salt-Proportions, with the Total Concentrations and Other Conditions Alike*

Following the preliminary work recorded in the previous section of this paper, wheat plants were grown in a complete series of sand cultures, to each pot of which was added, at 3-day intervals, a three-salt nutrient solution. The method by which the old solution was withdrawn and the fresh solution added to the pots has already been described. In this series, 36 cultures were employed, each of which received at the end of successive 3-day periods a culture solution having a total osmotic concentration of 1.75 atmospheres. The solution supplied to each particular culture differed, however, from that supplied to the other cultures in the series, with respect to the proportions of the three main component salts, monopotassium phosphate, calcium nitrate, and magnesium sulphate.

TABLE III

DRY WEIGHTS OF TOPS OF WHEAT GROWN FOR 20 DAYS IN SAND, WITH THREE-SALT SOLUTIONS VARYING IN TOTAL CONCENTRATION FROM 0.2 TO 1.8 ATMOSPHERES

Series C, conducted from April 28 to May 18, 1915

Culture No.	Total Concentration of Solution	Amount of Molecular Solution Required per Liter of Nutrient Solution			Dry Weights	
		KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	Absolute	Relative to Culture 1
	atm.	c.c.	c.c.	c.c.	gm.	
[1]0A	H_2O	....	....	....	.1547	....
0B	H_2O	....	....	....	.2260	....
1A	0.2	1.8	1.0	2.1	.4580	[2]1.00
1B	0.2	1.8	1.0	2.1	.4526	
2A	0.6	5.4	3.0	6.3	.6234	[2]1.32
2B	0.6	5.4	3.0	6.3	.5804	
3	1.0	9.0	5.0	10.5	.7910	1.73
4	1.4	12.6	7.0	14.7	.9368	2.06
5	1.8	16.2	9.0	18.9	.9180	2.00

[1] Culture number 0 received only distilled water in this series. Cultures 0, 1 and 2 were in duplicate, each pair being indicated by A and B.
[2] Mean from two cultures.

The method of calculation by which the partial osmotic concentration and the volume-molecular concentration of each component salt in mixtures such as this, with a fixed total osmotic concentration, has been discussed by Tottingham (40, p. 177-182, 192), in connection with his four-salt solutions. In calculating the amount of each salt required to produce the total concentration required by the series (1.75 atm.) it was assumed that the degree of ionization of each salt is independent of the presence of the other two salts. In other words, the assumption was here made that each of the three salts would behave, in the presence of the other two, in the same manner as it would if dissolved in distilled water. The lowering of the freezing-point was determined by Shive (36) for each

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one of the three-salt solutions employed by him, and it appeared from these determinations that the freezing-point lowerings were approximately the same for all of the solutions of his optimal series. Since Shive's series of optimal solutions are the ones here employed, it may safely be concluded that the *initial* total osmotic concentration of the nutrient solutions used in these sand cultures very closely approximated 1.75 atmospheres.

Table IV gives the volume-molecular concentrations of each salt required to produce from 1 tenth to 8 tenths of the total osmotic concentration for the various solutions in the series under consideration, these being taken from Shive's Table I (36, p. 339). To determine the volume molecular partial concentration of any given salt in any solution of this series it is only necessary to find, in the first column of this table, the number of tenths of the total concentration to be assigned to that particular salt, and then to take from the proper column the volume molecular concentration given opposite this number.

TABLE IV

VOLUME-MOLECULAR PARTIAL CONCENTRATIONS REQUIRED TO PRODUCE FROM FROM 1 to 8 TENTHS OF THE TOTAL OSMOTIC CONCENTRATION FOR A SERIES OF SOLUTIONS HAVING A TOTAL CONCENTRATION OF 1.75 ATMOSPHERES

Tenths of Total Concentration	Partial Concentrations in Gram Molecules per Liter		
	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$
1	0.0036	0.0026	0.0050
2	0.0072	0.0052	0.0100
3	0.0108	0.0078	0.0150
4	0.0144	0.0104	0.0200
5	0.0180	0.0130	0.0250
6	0.0216	0.0156	0.0300
7	0.0252	0.0182	0.0350
8	0.0288	0.0208	0.0400

For convenience in designating the individual cultures and to give clearness to the following discussions, the cultures may be arranged on an equilateral triangular diagram, as was done by Shive for his similar series. This diagram is shown in figure 2, and in another form in figures 3, 5 and 8. Similar graphic schemes have been used extensively in chemical interpretation, and have been employed by Schreiner and Skinner (33, 34) and by Tottingham (40), as well as by Shive (38) and by Harris (13). The individual cultures are represented by circles, and it will be observed that the diagram has eight rows, the lower one of which contains eight individual cultures. Proceeding upward each row has one culture less than the one below it, and the eighth row contains but a single culture. The employment of shaded segments to the various partial osmotic concentrations of the three salts, in each of the 36 cultures under consideration, is an adaptation of the scheme employed by Harris (13) in his study of the alkali salts in soils. The unshaded segment in each cir-

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cle represents the number of tenths of the total osmotic concentration derived from calcium nitrate; the segment marked by small crosses represents the number of tenths derived from mono-potassium phosphate; and the stippled segment indicates the number of tenths due to magnesium sulphate. The system of numbering the individual cultures, and the corresponding nutrient solutions, is the same in figure 1 as that employed by Tottingham (40, p. 194) and by Shive (38, p. 341). Proceeding from the base to the apex of the triangle, the rows are numbered from R1 to R8, while the individual cultures in each row are numbered from left to right. For example, the fourth culture from the left in the second row from the base is designated R2 C4, and similarly the second culture in the seventh row is R7 C2.

The diagram of figure 2 shows that all of the solutions represented as in the first row have approximately 1 tenth of their total osmotic concentration from mono-potassium phosphate, those in the second row 2 tenths, this amount increasing by increments of 1 tenth from row to row, until the apex of the triangle is reached, at which point the single culture in row 8 has 8 tenths of its total concentration due to mono-potassium phosphate. As indicated by the shading, the first culture, at the *left* in each row, has 1 tenth of its total concentration due to calcium nitrate, and this partial concentration increases regularly by increments of 1 tenth until the opposite side of the diagram is reached. In a similar manner the osmotic partial concentrations of magnesium sulphate increase from *right* to *left* in each row. The circle occupying the position R1 C2 has 1 tenth of its total area unshaded, 2 tenths marked by crosses, and the remaining 7 tenths stippled, thus indicating that the solution used for this culture had the osmotic proportions of 1 tenth mono-potassium phosphate, 2 tenths calcium nitrate and 7 tenths magnesium sulphate. Throughout this paper the individual cultures will be designated by the row number and by the position occupied in the row, using the nomenclature employed by previous writers. The partial volume-molecular concentration of each salt in each of the 36 solutions is given in Table V, together with the corresponding values of the three cation ratios. Each solution has a total osmotic concentration of 1.75 atmospheres.

Discussion of Results

I Introductory

The series of cultures which are now to be considered were grown in sand for a period of 24 days extending from May 15 to June 8, 1915. The wheat used was of the Fulcaster variety, from the same lot as was used by Tottingham (40) and by Shive (38), in their water cultures. As has already been stated, the methods employed for the germination of the seed and for the manipulation of the solutions were the same as those described by Shive (38), with such modifications as were made necessary by the employment of sand instead of water cultures. A detailed ac-

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count of the method employed in the preparation of the sand and the manipulation used to secure a renewal of the solution at regular intervals has already been given.

TABLE V

PARTIAL VOLUME-MOLECULAR CONCENTRATION OF EACH OF THE SALTS IN EACH OF THE 36 THREE-SALT SOLUTIONS EMPLOYED FOR WHEAT IN SAND CULTURES; ALSO, THE THREE VALUES OF THE CATION RATIOS FOR EACH SOLUTION

Total concentration of each solution 1.75 atmospheres

Solution Number	Partial Solutions in Gram-Molecules per Liter			Cation Ratio Values[1]		
	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	Mg/Ca	Mg/K	Ca/K
R1 C1	.0036	.0026	.0400	15.40	11.10	0.72
C2	.0036	.0052	.0350	6.74	9.72	1.44
C3	.0036	.0078	.0300	3.85	8.34	2.16
C4	.0036	.0104	.0250	2.40	6.94	2.88
C5	.0036	.0130	.0200	1.54	5.55	3.60
C6	.0036	.0156	.0150	0.96	4.17	4.33
C7	.0036	.0182	.0100	0.55	2.78	5.04
C8	.0036	.0208	.0050	0.24	1.39	5.77
R2 C1	.0072	.0026	.0350	13.46	4.86	0.36
C2	.0072	.0052	.0300	5.77	4.17	0.72
C3	.0072	.0078	.0250	3.21	3.47	1.08
C4	.0072	.0104	.0200	1.92	2.77	1.44
C5	.0072	.0130	.0150	1.15	2.08	1.80
C6	.0072	.0156	.0100	0.64	1.38	2.16
C7	.0072	.0182	.0050	0.27	0.69	2.52
R3 C1	.0108	.0026	.0300	11.53	2.78	0.24
C2	.0108	.0052	.0250	4.81	2.32	0.48
C3	.0108	.0078	.0200	2.53	1.85	0.72
C4	.0108	.0104	.0150	1.44	1.39	0.96
C5	.0108	.0130	.0100	0.77	0.98	1.20
C6	.0108	.0156	.0050	0.32	0.46	1.44
R4 C1	.0144	.0026	.0250	9.61	1.74	0.18
C2	.0144	.0052	.0200	3.85	1.39	0.36
C3	.0144	.0078	.0150	1.92	1.04	0.50
C4	.0144	.0104	.0100	0.96	0.69	0.72
C5	.0144	.0130	.0050	0.38	0.35	0.90
R5 C1	.0180	.0026	.0200	7.69	1.10	0.14
C2	.0180	.0052	.0150	2.88	0.83	0.29
C3	.0180	.0078	.0100	1.28	0.56	0.43
C4	.0180	.0104	.0050	0.48	0.28	0.58
R6 C1	.0216	.0026	.0150	5.77	0.69	0.12
C2	.0216	.0052	.0100	1.92	0.46	0.24
C3	.0216	.0078	.0050	0.64	0.23	0.36
R7 C1	.0252	.0026	.0100	3.85	0.40	0.10
C2	.0252	.0052	.0050	0.96	0.20	0.20
R8 C1	.0288	.0026	.0050	1.92	0.17	0.09

[1] These ratio values are based on the supposition that the salts are completely ionized, so that all the Mg, etc., in the solution is regarded as being in the form of Mg, etc., ions. This is, of course, not strictly true, but as the ions are absorbed by the plant more ions should be formed, so that eventually all of these atoms should be available as ions.

A continuous record of the temperature changes during the period of this series was secured by means of a thermograph. The highest temperature recorded was 34° C. (on May 22 and 25) and the lowest was 9° C.

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(on May 18 and 20). A cylindrical porous-cup atmometer, which was used to indicate the variations in the evaporating power of the air during the period in question, gave a daily mean water loss of 7.6 c.c., a maximum for a 24-hour period of 13.6 c.c. (on June 5), and a minimum of 1.4 c.c. (on May 30), with a total loss of 182 c.c. for the entire period of 24 days. These readings are all corrected to the Livingston standard cylindrical cup (21) by multiplying the actual readings by the coefficient furnished with the instrument.

In the following sections will be found a discussion of the physiological effects upon the wheat plants, produced by the various solutions, with their different salt-proportions, and a comparison of these results from sand cultures with those obtained by Shive from his corresponding water cultures, and also with Shive's results from his sub-optimal series, in which the total osmotic concentration was 0.1 atmosphere. The behavior of the plants in the different cultures will be compared with reference to the dry weights of tops and of roots and with respect to the relative amounts of water transpired during the growth period.

II. Dry Weights

(a) Method Employed in Harvesting

At the end of the growth period the wax seal was removed from the surface of the sand and the contents of the culture pot were carefully transferred to a coarse sieve having approximately 10 meshes to the inch. By means of a gentle stream of water the sand was then washed down through the sieve, leaving the plants and roots behind. Tho tops of the plants were severed from the roots just above the remnant of the seed and then dried in an electric oven at 80° C. for a period of 24 hours, after which they were dried to constant weight at an oven temperature of approximately 102° C. Since it was impossible to wash the sand entirely free from the roots, a different method of procedure was necessary in order to obtain the dry weight of these subterranean parts. Without attempting to remove the last traces of sand, the roots were transferred from the sieve to a piece of paper and allowed to attain an air-dry condition, after which they were dried in the oven, just as in the case of the tops, until the oven-dry weights were obtained. These included, for each lot of roots, the weight of the adhering sand as well as the weight of the roots themselves. To correct this error the oven-dried roots were ignited in porcelain crucibles until all the organic matter had been destroyed. The loss on ignition of the samples was recorded as representing the approximate dry weight of the roots, upon the assumption that the sand adhering to the roots was all non-combustible and therefore suffered no loss in the ignition process. For practical purposes the small amount of ash resulting from the ignition of the root tissues may be neglected, especially since the relative weights would be affected only by the difference between the weights of the ash from the various individual cultures.

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(b) Presentation of Data

In Table VI are presented the transpiration data for the individual cultures, together with the dry weights of tops and of roots. The transpiration data include (1) the actual water loss, in cubic centimeters, of

TABLE VI

TRANSPIRATION RECORD AND DRY WEIGHTS OF TOPS AND OF ROOTS FOR WHEAT GROWN 24 DAYS IN SAND CULTURES, SUPPLIED WITH THREE-SALT SOLUTIONS, ALL HAVING A TOTAL OSMOTIC CONCENTRATION OF 1.75 ATMOSPHERES, BUT DIFFERING FROM EACH OTHER IN THE PROPORTION OF THE THREE SALTS EMPLOYED

Culture Number	Transpiration (6 Plants)		Dry Weight of Tops[1] (6 Plants)		Dry Weight of Roots[1] (6 Plants)	
	Total Water Loss	Relative to R1 C1 as Unity	Absolute	Relative to R1 C1 as Unity	Absolute	Relative to R1 C1 as Unity
	gm.		gm.		gm.	
R1 C1	175.3	1.00	0.6412	1.00L	.1180	1.00L
C2	245.7	1.41	0.9504	1.48	.2118	1.79
C3	273.3	1.56	1.0723	1.67	.1672	1.41L
C4	271.2	1.55	1.1276	1.75H	.1936	1.63L
C5	316.2	1.80	1.0612	1.65	.2171	1.83
C6	321.6	1.83	1.1882	1.85H	.2174	1.84
C7	341.2	1.95	1.2181	1.90H	.2318	1.95
C8	339.9	1.94	1.2811	2.00H	.2168	1.83
R2 C1	205.8	1.17	0.6285	0.95L	.1844	1.55L
C2	248.3	1.42	0.8474	1.32	.2134	1.88
C3	303.8	1.73	1.0445	1.68	.2210	1.88
C4	353.7	2.00	1.2770	2.00H	.2652	2.24H
C5	339.5	1.94	1.1428	1.78H	.3166	2.67H
C6	321.8	1.83	1.1420	1.78H	.2597	2.19
C7	391.0	**2.23H**	1.4660	**2.29H**	.3333	**2.81H**
R3 C1	236.3	1.34	0.7080	1.11L	.2828	2.38H
C2	306.0	1.75	1.0358	1.62	.2969	2.50H
C3	263.8	1.50	0.9072	1.43	.2471	2.08
C4	312.0	1.78	1.0140	1.58	.2642	2.23H
C5	314.8	1.79	1.0810	1.71	.2786	2.35H
C6	336.1	1.92	1.0972	1.73H	.2077	1.75
R4 C1	192.2	1.09	0.5201	0.86L	.2531	2.13
C2	308.3	1.75	1.0330	1.62	.2969	2.50H
C3	260.6	1.50	0.8310	1.30L	.2316	1.95
C4	327.9	1.87	1.1033	1.72	.2630	2.22
C5	302.5	1.72	0.9848	1.54	.2440	2.06
R5 C1	237.6	1.35	0.6822	1.07L	.2440	2.06
C2	251.4	1.44	0.7790	1.23L	.1786	1.50L
C3	251.6	1.44	0.7763	1.21L	.1794	1.51L
C4	289.4	1.65	0.8912	1.39	.1549	1.31L
R6 C1	261.3	1.50	0.8489	1.32	.1527	1.30L
C2	306.7	1.75	0.9151	1.43	.1730	1.46L
C3	358.1	2.05	0.9460	1.48	.2598	2.19
R7 C1	208.6	1.19	0.6285	0.95L	.2044	1.72
C2	286.8	1.65	0.9540	1.50	.2266	1.91
R8 C1	277.6	1.60	0.8466	1.32	.2669	2.25H

[1]The best nine cultures are marked H, while the poorest nine are marked L.

each individual culture for the entire growth period and (2) these same quantities expressed as relative to the loss from culture R1 C1, taken as unity. In the dry weight columns are recorded (1) the absolute dry

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weights, in grams, of both tops and roots separately and (2) the weights of tops and of roots relative to those of culture R1 C1, taken as unity. The maximum transpiration and the highest yields of tops and roots are here indicated by black-face type. A discussion of the transpiration data will be presented under a separate heading, following the discussion devoted to a comparison of the results obtained in the sand cultures with those secured in solution cultures.

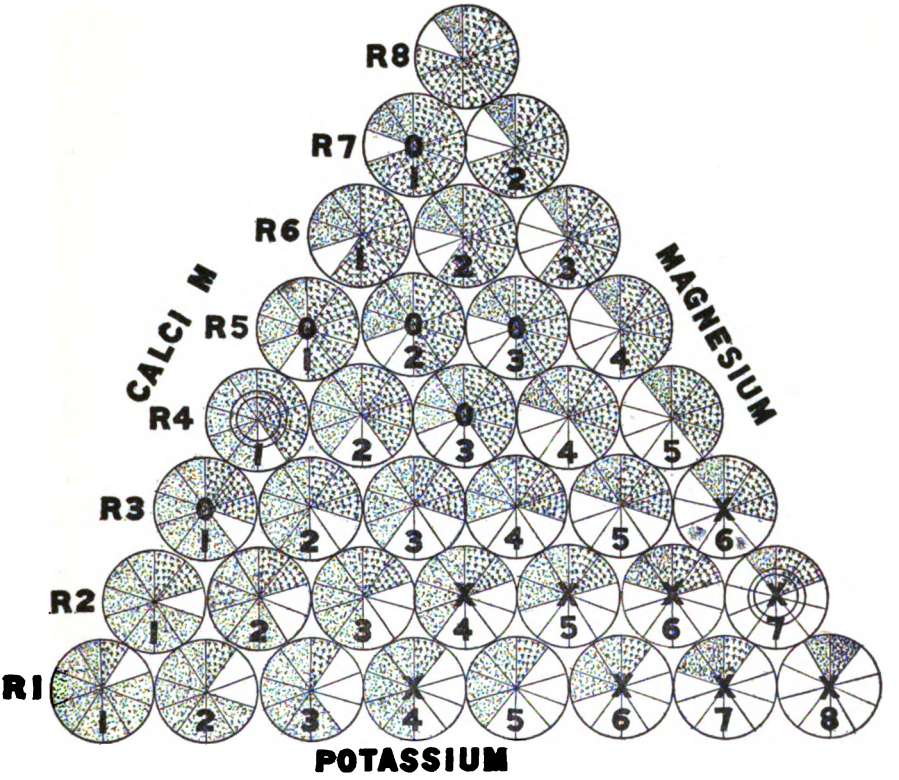

Fig. 2.—Triangular diagram showing the arrangement of the sand cultures with respect to the partial concentrations of the three salts employed. Unshaded segments represent the proportions of $Ca(NO_3)_2$: stippled segments the $MgSO_4$: and the segments shaded with crosses the KH_2PO_4. The best nine cultures are marked X, while the poorest nine are marked by O.

In order to study better the relative growth rates, the entire series of 36 cultures are divided into three groups, (1) a lower one-fourth composed of the 9 cultures giving the lowest yields (either tops or roots), (2) an upper one-fourth composed of the 9 cultures giving the highest yield values, and (3) a medium one-half which comprised the remaining cultures. To facilitate the comparison, the solution cultures (38) were treated in exactly the same manner. In Tables VI and VII the relative

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yields are marked with an L if they lie in the low yield group and with an H if they lie within the high yield group. The cultures with high and with low values for weights of tops are shown on the diagram of figure 2. These, and similar groups of cultures, are to be found on the diagrams of figures 3 and 5, and will be referred to, always, as the *best* nine and the *poorest* nine.

III. A Comparison of the Results from Sand Cultures with those from Solution Cultures

(a) Dry Weight of Tops

As has been mentioned, the results here brought forward were secured by using seed from the same lot as that from which Shive's seed was selected, and it thus seems desirable to compare the growths secured with these sand cultures with those obtained by Shive with solution cultures.

The second and third columns of Table VII present the relative dry weights of tops and of roots for the various sand cultures here employed, which were all supplied with nutrient solutions of the same total osmotic concentration, but of different salt-proportions, as already described. In columns 4 and 5 of this table are given the relative dry weights of tops and of roots secured by Shive in his sub-optimal cultures, all these solutions having a total osmotic concentration of 0.1 atmosphere. In the two columns at the right of the table are given the relative dry weights of tops and of roots for Shive's optimal series, with solutions having the same total osmotic concentration (1.75 atmospheres) as those employed in the sand cultures of the present study. The *actual* dry weights, in grams, of culture R1 C1 are given in parentheses directly below the relative weight values. The actual dry weight of any culture may be obtained by multiplying the relative weight by the actual weight of culture R1 C1 as given in the same column. Shive's supra-optimal series of cultures, with total concentration of 4.0 atmospheres, is not here considered.

To facilitate a general comparison of these three sets of cultures, the relative yields of tops are graphically shown in the triangular diagrams of figure 3, where A represents the sand cultures and B and C represent Shive's sub-optimum and optimum series, respectively. The areas of high yields are here indicated by crosses and those of low yields are shown by small circles, as was done by Shive. In each diagram the culture giving the highest yield is indicated by a large X.

It is readily apparent that there is a marked similarity, with respect to the location of the area of the poorest growth of tops, between the diagram for the sand cultures (1.75 atmospheres, total concentration, fig. 3, A) and that representing Shive's sub-optimal series (0.1 atmosphere, total concentration, fig 3, B). In fact, all three of the diagrams show a mark-

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ed similarity in this respect. The culture giving the highest yield of tops in the sand series is R2 C7, which is characterized by having 2 tenths of

TABLE VII

COMPARISON OF THE RELATIVE DRY WEIGHTS OF TOPS AND OF ROOTS OF WHEAT GROWN IN SAND CULTURES WITH CORRESPONDING DATA FOR WHEAT GROWN IN SOLUTION CULTURES

Culture Number	Sand Cultures (McCall) Total Concentration of Solution 1.75 atm.		Sub-optimal and Optimal Solution Cultures (Shive, 1915)			
			Total Concentration 0.1 atm.		Total Concentration 1.75 atm.	
	Relative Dry Weight[1]		Relative Dry Weight		Relative Dry Weight	
	Tops	Roots	Tops	Roots	Tops	Roots
R1 C1	1.00L	1.00L	1.00L	1.00	1.00L	1.00
	(0.6412)	(0.1186)	(0.2601)	(0.1036)	(0.4104)	(0.1058)
C2	1.48	1.79	1.22	1.05	1.19	1.11H
C3	1.67	1.41L	1.25	0.99	1.20	0.93
C4	1.75H	1.63L	1.30	0.91	1.17L	1.07H
C5	1.65	1.83	1.24	0.86L	1.26	0.99
C6	1.85H	1.84	1.38	0.98	1.16L	1.03
C7	1.90H	1.95	1.25	0.78L	1.11L	1.01
C8	2.00H	1.84	1.23	0.80L	1.17	0.95
R2 C1	0.95L	1.55L	1.03L	1.19	1.03L	0.96
C2	1.32	1.88	1.20L	1.04	1.14L	1.05H
C3	1.68	1.88	1.39	0.93	1.25	0.93L
C4	2.00H	2.24H	1.48H	0.94	1.27H	0.95
C5	1.78H	2.67H	1.40	0.77L	1.18	0.90L
C6	1.78H	2.19	1.43H	0.76L	1.22	0.98
C7	2.29H	2.81H	1.39	0.80L	1.23	1.04
R3 C1	1.11L	2.38H	1.11L	1.39H	1.15L	1.02
C2	1.16	2.50H	1.28	1.19H	1.24	1.07H
C3	1.43	2.08	1.42H	1.00	1.36H	1.07H
C4	1.58	2.23H	1.57H	0.94	1.28H	0.95
C5	1.71	2.35H	1.52H	0.82L	1.25	0.92L
C6	1.73H	1.75	1.35	0.91	1.27H	0.93L
R4 C1	0.86L	2.13	1.00L	1.31H	1.12L	1.04
C2	1.62	2.50H	1.21	1.22H	1.28H	1.10H
C3	1.30L	1.95	1.41	1.06	1.26	0.91L
C4	1.72	2.22	1.57H	0.89	1.27	0.91L
C5	1.54	2.06	1.65H	0.89L	1.30H	1.04
R5 C1	1.07L	2.06	1.09L	1.30H	1.19	1.07H
C2	1.23L	1.50L	1.47H	1.18	1.39H	1.08H
C3	1.21L	1.51L	1.41	0.90	1.24	0.91L
C4	1.39	1.31L	1.32	0.72L	1.28H	1.03
R6 C1	1.32	1.30L	1.10L	1.31H	1.17	1.06H
C2	1.43	1.46L	1.29	1.07	1.19	0.91L
C3	1.48	2.19	1.44H	1.02	1.21	0.87L
R7 C1	0.95L	1.72	1.11L	1.36H	1.16L	1.03
C2	1.50	1.91	1.29	1.28H	1.31H	0.98
R8 C1	1.32	2.25H	1.10L	1.35H	....	....
Check[2]	0.52	1.78	....	....	....	....

[1] The dry weight of culture R1 C1 is always taken as unity and the other weights are expressed in terms of this value. The actual dry weight of culture R1 C1 is given in parentheses, in grams. The best nine cultures are marked H, while the poorest are marked L.

[2] Check received only distilled water.

its total osmotic concentration derived from mono-potassium phosphate, 7 tenths from calcium nitrate and 1 tenth from magnesium sulphate. This

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culture gave a yield of dry tops 129 per cent greater than that of R1 C1. The culture giving the highest yield of tops in Shive's sub-optimal solution cultures was R4 C5, its yield being 65 per cent higher than that from culture R1 C1 in the same series. This culture solution is characterized

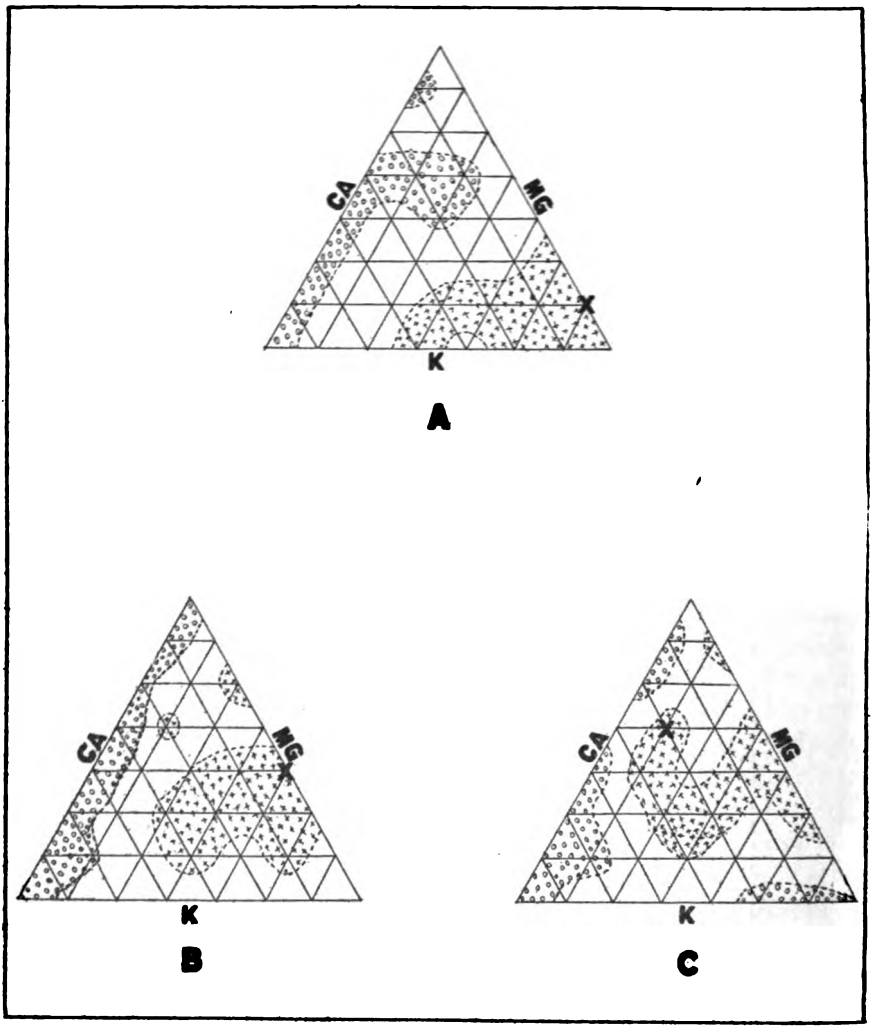

Fig. 3.—Triangular diagrams showing areas of high and of low yield of tops. A, sand cultures; B, Shive's sub-optimal, and C, Shive's optimal solution cultures.

by having 4 tenths of its total osmotic concentration derived from monopotassium phosphate, 5 tenths from calcium nitrate and 1 tenth from magnesium sulphate. In Shive's optimal series (1.75 atmospheres) the highest dry weight of tops was obtained from culture R5 C2, and this

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yield was 39 per cent higher than that from culture R1 C1 in the same series. This solution is characterized by having 5 tenths of its total osmotic concentration due to mono-potassium phosphate, 2 tenths to calcium nitrate, and 3 tenths to magnesium sulphate. These data are summarized in Table VIII.

TABLE VIII

OSMOTIC PROPORTIONS OF THE THREE-SALT SOLUTIONS GIVING THE BEST GROWTH OF TOPS IN THE SAND CULTURE SERIES (McCALL) AND IN THE SUB-OPTIMAL AND OPTIMAL SOLUTION SERIES (SHIVE)

Series	Relative Dry Weight of Tops	Total Concentration atm.	Osmotic Proportions, in Tenths, of of Total Concentration		
			KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$
Sand Cultures (McCall)	2.29	1.75	2	7	1
Sub-optimal (Shive) ..	1.65	0.10	4	5	1
Optimal (Shive)	1.39	1.75	5	2	3

A comparison of the best sand culture (total concentration 1.75 atmospheres) with the best solution culture of the sub-optimal series (total concentration 0.1 of an atmosphere) brings out the fact that the osmotic concentration ratio of magnesium sulphate to mono-potassium phosphate plus calcium nitrate is the same for both cultures, namely 1:9. The ratio of magnesium sulphate to calcium nitrate is 1:7 for the sand culture and 1:5 for the solution culture. The greatest difference between the relative proportions of the salts employed is shown by the ratio of calcium nitrate to mono-potassium phosphate, this ratio being 7:2 for the sand and 5:4 for the solution culture.

A comparison of the best sand culture with the solution culture giving the best growth of tops in Shive's optimal series, having the same total osmotic concentration (1.75 atm.) brings out some surprising results. In these two cultures there is a marked difference in salt proportions. Shive's best solution in his optimal series is characterized by a value of 3:7 for the osmotic ratio of magnesium sulphate to calcium nitrate plus mono-potassium phosphate; while in the best culture of the sand series and the best in the sub-optimal solution series this ratio is 1:9, as has already been stated. The most striking difference between the best culture of the sand series and the best of Shive's optimal water-culture series is found, however, in the relation of magnesium to calcium. In the best solution of Shive's series the osmotic ratio of magnesium sulphate to calcium nitrate is 3:2, while for the best culture of the sand series this ratio is 1:7. The osmotic ratio of the calcium salt to the potassium salt is also markedly different in these two cases, being 2:5 for the best solution culture and 7:2 for the best sand culture.

While it appears to be impossible to draw any definite conclusions from a detailed study of the characteristics of the solutions that produced

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the best growth of tops in the three series it is important to note (1) that the results secured in the sub-optimal solution series are more nearly like those from the sand series than are the results obtained from the optimal solution series with the same total osmotic concentration; (2) that there is a marked difference between the solutions producing the best growth of tops in sand and those giving the best growth of tops in solution cultures, with respect to the osmotic proportions of the three salts employed. Attention is called to the fact that the improvement in growth of tops as we proceed to the right from the left margin of the triangle is very much more marked in the sand than in the solution series. This is brought out in a striking manner by the graphs of figure 4, which show the variations in the yield of tops of the individual cultures in the sand series and in Shive's optimal and sub-optimal solution series.

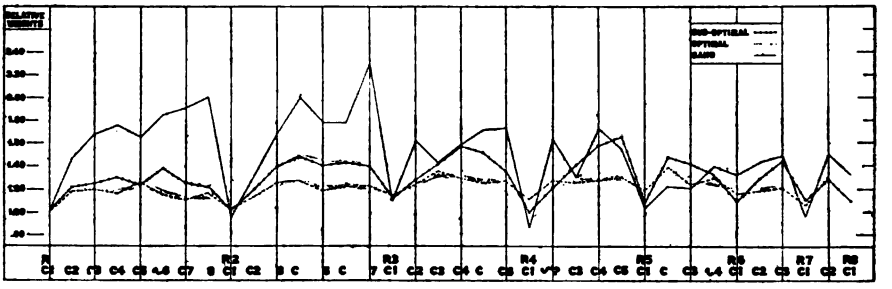

Fig. 4.—Relative dry weights of cultures grown in sand and in solution cultures of sub-optimal and of optimal total concentrations.

The most striking feature of these graphs is the regularity with which the one for the sand series of cultures intersects those for the two solution series. It will be seen that this intersection occurs always at culture No. 1 of each row, as represented on the triangular diagram, with the exception of R6 C1 and of R8 C1. A possible explanation of the phenomenon thus indicated is offered by the known selective adsorptive property of sand and other finely divided substances.

As early as 1866, Frank (11), studied the retention of potassium chloride by the soil, finding that soil has the power of absorbing, or removing from solution, considerable amounts of this salt. Subsequent investigations have shown that this power to remove salts from solution is also possessed by other finely divided substances that are chemically inert, such as charcoal and pulverized silica. More recent work has not only confirmed these early observations, but has brought out the fact that finely divided substances may exercise a selective action with respect to the solution with which they are brought into contact. In some cases the effect of this selective

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action is to remove one ion of the salt more rapidly than the other, leaving the solution alkaline or acid, depending upon which ion is removed to the greater extent.[1] It has also been found that, in certain cases, the selective adsorption increases in amount with the concentration of the solution, up to a certain maximum, and then remains constant with still further increase in concentration.

To employ these considerations in an attempt to explain the physiological phenomena in question, it may be supposed that the poor growth of all of the C1 cultures, whether in sand or solution, is due to a deficiency of calcium nitrate together with the accompanying excess of magnesium sulphate, thus leaving out of account, for the present, row 6 and the single culture at the apex of the diagram. In the solution cultures, as we pass from C1 to C2, in each row, the proportion of calcium nitrate to magnesium sulphate becomes slightly more favorable, giving rise to a slight increase in the dry weight from C2 as compared with C1. It may also be supposed that the partial concentration of the magnesium salt is here the factor limiting the growth of the plants and that this salt antagonizes the NO_3 radical, thus preventing the latter from exerting an accelerating influence on the growth rate. In the sand cultures it is possible that the NO_3 radical is not appreciably adsorbed by the sand, but that a part of the magnesium present in the system is so adsorbed, thus being prevented from active participation in the physiological processes of the plants.[2]

To go farther with this hypothesis, it may be supposed that this adsorption of magnesium is not sufficient, in the left cultures of the diagram, to allow the NO_3 ions to assert themselves by accelerating the growth of the plants. As we proceed toward the right, on the triangular diagram, the partial concentration of magnesium sulphate in the original solution decreases by increments of 1 tenth of the total concentration, from each culture to the next. Now, the magnesium sulphate actually free to affect the plants of the sand cultures is the amount of this salt in the original solution minus the amount that has been adsorbed by the sand, and it may thus be that the very marked progressive improvement in growth as we proceed from left to right across the diagram is due to a parallel increase in the partial concentration of the calcium nitrate accompanied by a corresponding decrease in the magnesium sulphate. This alteration in the salt-proportions (or ion-proportions) of the unadsorbed solution brought about by the selective adsorption of magnesium sulphate by the solid medium, may give rise to a better physiological balance than that which characterizes the unmodified solution.

[1] See in this connection: Gore, G. (12); Briggs, J. L. (3); Cameron, F. C., and Bell, J. M. (6); Parker, E. G. (29); Williams, A. M. (45); and McCall, A. G. (26).

[2] While no direct evidence bearing upon this point can be produced, it may be mentioned that Parker (29, p. 188) found that sodium nitrate in certain partial concentrations increased the adsorption, by the soil, of potassium chloride out of the same solution.

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(b) Dry Weight of Roots

The dry weights of roots are given in Table VII, in connection with the dry weights of tops. The graph presented in figure 6 shows the variation in dry weight of roots between the individual cultures. The marked characteristic of this diagram is the manner in which the graph makes a gradual rise to near the middle of the series and then gradually declines until near the end, where a slight rise again occurs.

The discussion of these data can best be presented by referring to the triangular diagram of figure 5A, in which the area of high relative values is indicated by crosses and that of low values is marked by circles. The relative dry weights of roots have a total range from 1.00 (culture R1 C1) to a maximum of 2.81 (culture R2 C7). A comparison of this diagram with figure 3A, giving the dry weights of tops, brings out the interesting fact that the culture showing the best growth of roots (culture R2 C7) is also the one that gave the highest yield of tops. This culture gave 129 per cent greater yield of tops and 181 per cent greater yield of roots than did culture R1 C1. No such correlation is apparent, however, between the culture giving the poorest yield of tops and the one showing the poorest root development.

Considering, now, the areas of high and of low root yields, it will be seen that an area of high yields (2.22 to 2.81) extends nearly across the entire width of the triangle, in a regular belt beginning at row 2 on the right and passing obliquely upward to the center of the triangle (where a slight break occurs) thence to the right margin at row 3. In a similar manner an area of low yields (1.30 to 1.51) extends across the entire width of the triangle, occupying a position above the area of high yields and being confined to rows 5 and 6. Two small areas of low yields are shown at the lower margin of the diagram, each of which includes two cultures.

The solution giving the best growth of roots, as in the case of tops, is characterized by having 2 tenths of its total osmotic concentration derived from mono-potassium phosphate, 7 tenths from calcium nitrate and the remaining 1 tenth from magnesium sulphate.

The culture giving the poorest growth of roots is culture R1 C1, which lies outside of the main area of low root yields. This culture has 1 tenth of its total osmotic concentration due to mono-potassium phosphate, 1 tenth to calcium nitrate, and the remaining 8 tenths to magnesium sulphate.

A comparison of this diagram with the corresponding diagrams (figure 5, B and C) of Shive's solution cultures serves to bring out the fact that the areas of highest and of lowest yields of roots extend in narrow strips across the triangular diagram for the sand cultures in a direction almost at right angles to the direction taken by the corresponding areas on the diagrams representing the solution cultures with total concentra-

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tion of 0.1 and 1.75 atmospheres. A comparison of the root yields obtained from the sand cultures with those obtained from the solutions of the same total concentration as was used in the sand series (1.75 atm.) and also with those obtained from solutions having a concentration of 0.1 atmosphere fails to reveal any further generalization that might be of interest or value in this connection.

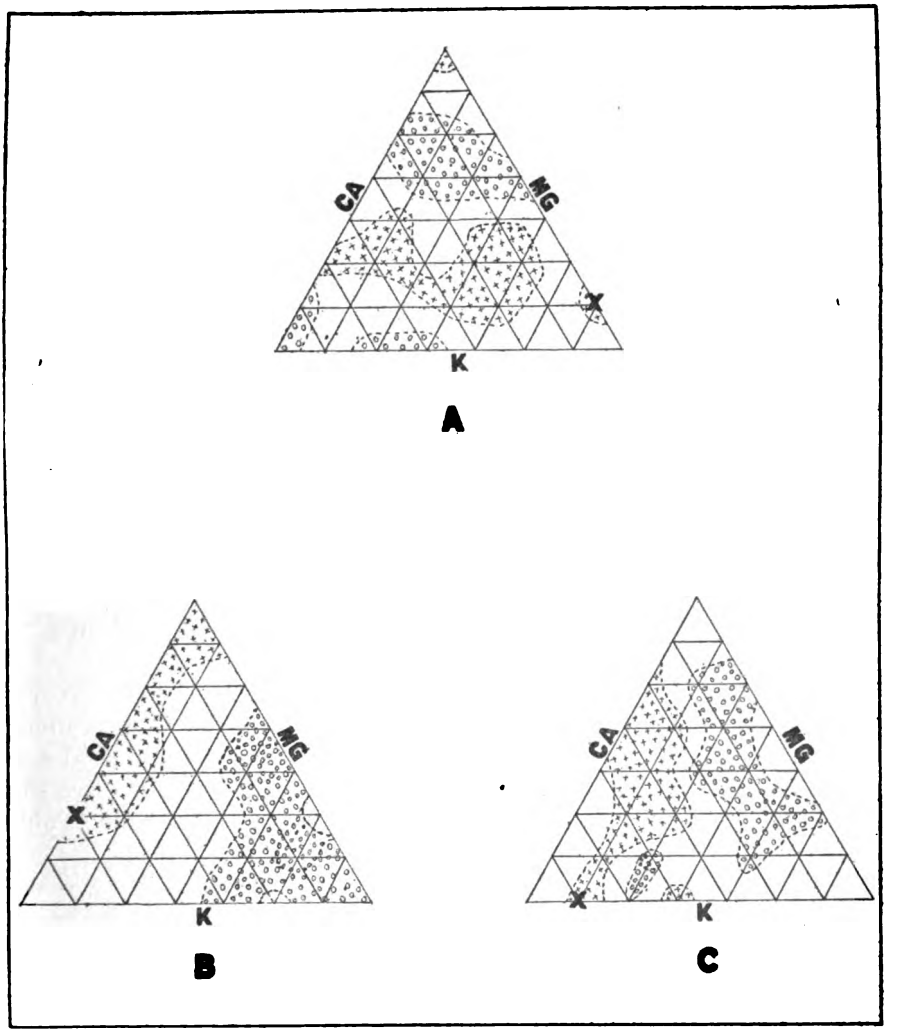

Fig. 5.—Triangular diagram showing areas of high and of low yield of roots. A, sand cultures; B, Shive's sub-optimal, and C, Shive's optimal solution cultures.

IV. Relation of Top Yields to Cation Ratio Values

Introduction. The dissociation of the three salts employed in these culture solutions gives rise to three kinds of cations (Ca, K and Mg) and three kinds of anions (NO_3, PO_4 and SO_4), if we assume a com-

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plete ionization of the salts and disregard the H ions and the HPO_4 ions produced by the dissociation of mono-potassium phosphate. Since these chemical units also appear to function as units in the metabolic processes of the plant, and since absorption of any ion by the plant should lead to further production of that ion in the solution, until all of the molecules in question, originally present, had been separated into ions, it is of interest to study the relation between the ion ratios and the yield of tops, somewhat as was done by Shive in his study of solution cultures corresponding to the sand cultures here considered.

As the last mentioned writer has pointed out, one of these three-salt solutions may be defined by the three cation ratios, Mg/Ca, Mg/K and Ca/K, for the values of any other of the possible ion ratios may be found directly from these three. The cation ratio values alone will therefore be considered in this discussion.

It sould be noted at once that the ionic ratios may be very greatly modified by the presence of the sand, which, through its adsorptive power may not only markedly reduce total concentration of the original solution, but may also alter the original relative proportions of the component salts and ions. As has been emphasized above, the adsorptive action may fall more heavily on some of the ions than upon others, so that the unadsorbed solution remaining in the sand of the culture pots may be characterized by very different molecular and ionic ratios from those of the original solutions with which the pots were first saturated. It is highly probable that selective adsorption plays an important rôle in bringing about the physiological differences to be observed between the sand cultures of the present paper and the corresponding solution cultures of Shive's study.

Dry weight of tops. Range of cation ratios for the best nine cultures. Table IX presents the cation ratio values for each of the nine solutions (of the sand cultures and of the sub-optimal and optimal) that produced the highest yield of tops. The best nine cultures, in each case, are the ones marked H in Tables VI and VII. The cultures are here (Table IX) arranged in the descending order, on the basis of top yields, the one producing the highest yield in each series being placed at the head of the column. In the columns giving the cation ratios the minimum and the maximum values for these ratios are marked L and H, respectively. The total range in the magnitude of these ratio values is given at the bottom of the respective column.

It will be observed that the group of sand-culture solutions giving high yields of tops is characterized by a comparatively low range (2.16) in the Mg/Ca ratio value, extending from minimum of 0.24 to maximum of 2.40. This group of highest yielding cultures includes the lowest value of this ratio, but is restricted to the lower one-sixth of the total range of these ratio values. Both the Mg/K and the Ca/K ratio values show a

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much larger range than does the Mg/Ca ratio. From a consideration of these data it may be concluded that in sand cultures, such as were here employed, we may expect to find good growth of tops associated with Mg/Ca ratio values between 0.24 and 2.40; a range of Mg/K ratio values from 0.46 to 6.94; and a range of Ca/K ratio values from 1.44 to 5.77. The culture (R2 C7) giving the largest yield of tops is characterized by a low Mg/Ca ratio (0.46), a low Mg/K ratio value (0.69) and an intermediate value (2.52) for the Ca/K ratio.

TABLE IX

RANGE OF CATION RATIO VALUES AND RELATIVE DRY WEIGHTS OF TOPS OF THE BEST NINE CULTURES OF WHEAT GROWN IN SAND AND IN SOLUTION CULTURES

	Culture Numbers	Cation Ratio Values			Yield of Tops Relative to that of Culture R1 C1
		Mg/Ca	Mg/K	Ca/K	
Sand cultures supplied with a solution having a concentration of 1.75 atm.	R2 C7	0.27	0.69	2.52	2.29
	R1 C8	0.24L	1.39	5.77H	2.00
	R2 C4	1.92	2.77	1.44	2.00
	R1 C7	0.55	2.78	5.04	1.90
	R1 C6	0.96	4.17	4.33	1.85
	R2 C5	1.15	2.05	1.80	1.78
	R2 C6	0.64	1.38	2.16	1.78
	R1 C4	2.40H	6.94H	2.88	1.75
	R3 C5	0.32	0.46L	1.44L	1.73
Range		2.16	6.48	4.33	
Solution cultures of sub-optimal concentration 0.1 atm. (Shive)	R4 C5	0.38L	0.35	0.90	1.65
	R3 C4	1.44	1.39	0.96	1.57
	R4 C4	0.96	0.69	0.72	1.57
	R3 C5	0.77	0.93	1.20	1.52
	R2 C4	1.92	2.77H	1.44	1.48
	R5 C2	2.88H	0.83	0.29L	1.47
	R6 C3	0.64	0.23L	0.36	1.44
	R2 C6	0.64	1.39	2.16H	1.43
	R3 C3	2.56	1.85	0.72	1.42
Range		2.50	2.54	1.87	
Solution cultures of optimal concentration 1.75 atm. (Shive)	R5 C2	2.88	0.83	0.29	1.39
	R3 C3	2.56	1.85	0.72	1.36
	R7 C2	0.96	0.20L	0.20L	1.31
	R4 C5	0.33	0.35	0.90	1.30
	R4 C2	3.85H	1.39	0.36	1.28
	R5 C4	0.48	0.28	0.58	1.28
	R3 C4	1.44	1.39	0.96	1.28
	R3 C6	0.32L	0.46	1.44	1.27
	R2 C4	1.92	2.77H	1.44H	1.27
Range		3.53	2.57	1.24	

From a comparison of these ratio values with those obtained in the solution cultures, it will be seen that, with respect to the Mg/Ca ratio, there is substantial agreement, the range for the sand culture series being 2.16, that for Shive's solution culture series, with total osmotic concentration of 0.1 atm., 2.50, and for the solution cultures, with a total osmotic

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concentration of 1.75 atm., the range is 3.53. The range of the Mg/K ratio value is much greater for the sand culture series than for the solution culture series, both of which are characterized by a medium low range for this ratio. The range of the Ca/K ratio value for the sand cultures is very wide, and includes all possible values except a few of the very lowest. The solution culture series, on the contrary, show low ranges of this ratio and include neither the high nor any of the extremely low ratio values.

From these data it appears that there is substantial agreement between the three series with respect to the range of the Mg/Ca ratio of the solutions employed, but that no such agreement is to be found with respect to the other two cation ratios here considered, hence we may conclude that for the three-salt solution here employed the ratio of magnesium to calcium is more important than the ratio of magnesium to potassium or of calcium to potassium, in determining the best yield of tops. Attention is here directed to the fact that the first mentioned ratio is very closely related to the lime-magnesia ratio, so much discussed, and for this reason it will receive attention in a special chapter.

Dry weight of tops. Range of cation ratios for the poorest nine cultures. Table X shows the cation ratio values for each of the nine solutions (both sand and solution culture series) that produced the lowest yield of tops. The poorest nine cultures, in each case, are the ones marked L in Tables VI and VII. The cultures are here (Table X) arranged in the ascending order, the one producing the lowest yield in each series being placed at the head of the column. As in the previous section (Table IX), the minimum and the maximum values for the ratios are marked L and H, and the total range in the magnitude of these ratio values is given at the bottom of the respective column.

It will be observed that the group of sand-culture solutions giving the lowest yield of tops is characterized by a very wide range in the Mg/Ca ratio values, which include all but the very lowest values. The Mg/K ratios cover practically the entire range of values, while the Ca/K ratio shows a very low range, which is confined to the low values. The poorest individual culture is characterized by a high (9.61) Mg/Ca ratio, a low (1.74) Mg/K ratio, and a very low (0.18) Ca/K ratio. From a consideration of these data, it may be concluded, that in sand cultures such as were here employed, we may expect to find poor growth of tops associated with a very low ratio of calcium to potassium. It appears, further, that the ratios of magnesium to potassium and of calcium to potassium are not important factors in bringing about a *poor* growth of tops.

From a comparison of these ratio values with those obtained in the solution cultures it will be seen that with respect to the range in the Mg/Ca and the Mg/K ratio values there is a very close agreement, the range being very wide for all three of the series here considered. With

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respect to the range in the Ca/K ratios there is perfect agreement between the sand culture series (1.75 atm.) and the sub-optimal (0.1 atm.) solution culture series, the range in both cases being very narrow, but the optimal (1.75 atm.) solution culture series has a wide range for this ratio.

TABLE X

RANGE OF CATION RATIO VALUES AND RELATIVE DRY WEIGHTS OF TOPS OF THE POOREST NINE CULTURES OF WHEAT GROWN IN SAND AND IN SOLUTION CULTURES

	Culture Numbers	Cation Ratio Values			Yield of Tops Relative to that of Culture R1 C1
		Mg/Ca	Mg/K	Ca/K	
Sand cultures supplied with a solution having a concentration of 1.75 atm.	R4 C1	9.61	1.74	0.18	0.86
	R2 C1	13.46	4.86	0.36	0.95
	R7 C1	3.85	0.40L	0.10L	0.95
	R1 C1	15.40H	11.10	0.72H	1.00
	R5 C1	7.69	11.11H	0.14	1.07
	R3 C1	11.53	2.78	0.24	1.11
	R5 C3	1.28L	0.56	0.43	1.21
	R5 C2	2.88	0.83	0.29	1.23
	R4 C3	1.92	1.04	0.54	1.30
Range		14.12	10.70	0.62	
Solution cultures of sub-optimal concentration 0.1 atm. (Shive)	R1 C1	15.40H	11.10H	0.72H	1.00
	R4 C1	9.61	1.74	0.13	1.00
	R2 C1	13.46	4.86	0.36	1.03
	R5 C1	7.70	1.11	0.14	1.09
	R6 C1	5.77	0.69	0.12	1.10
	R8 C1	1.92L	0.18L	0.10L	1.10
	R3 C1	11.55	2.78	0.24	1.11
	R7 C1	3.85	0.40	0.10	1.11
	R2 C2	5.77	4.17	0.72	1.20
Range		13.48	10.92	0.62	
Solution cultures of optimal concentration 1.75 atm. (Shive)	R1 C1	15.40H	11.10H	0.72	1.00
	R2 C1	13.46	4.86	0.36	1.03
	R1 C7	0.55	2.78	5.04H	1.11
	R4 C1	9.61	1.74	0.18	1.12
	R2 C2	5.77	4.17	0.72	1.14
	R3 C1	11.55	2.78	0.24	1.15
	R1 C6	0.96L	4.17	4.32	1.16
	R7 C1	3.85	0.40L	0.10L	1.16
	R1 C4	2.40	6.96	2.88	1.17
Range		14.44	10.70	4.94	

Dry weight of roots. Range of cation ratios for the best nine cultures. In Table XI are presented the cation ratio values for the best nine cultures (both sand and solution culture series) based on the dry weight of roots. As in case of the dry weight of tops, the best nine cultures in every case are the ones marked H in Tables VI and VII, the order of arrangement and the numbering of the high and low ratio values being the same as that followed in Table IX. It will be seen that the group of

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sand culture solutions giving high yield of roots is characterized by a very wide range (11.26) for the Mg/Ca ratio values and medium low ranges (2.60 and 2.43, respectively) for the Mg/K and Ca/K ratios. The individual culture giving the largest weight of roots is characterized by very low values for both the Mg/Ca and the Mg/K cation ratios, and a medium value for the Ca/K ratio. The ratio ranges of the sand culture series

TABLE XI

RANGE OF CATION RATIO VALUES AND RELATIVE DRY WEIGHTS OF ROOTS OF THE BEST NINE CULTURES OF WHEAT GROWN IN SAND AND IN SOLUTION CULTURES

	Culture Numbers	Cation Ratio Values			Yield of Roots Relative to that of Culture R1 C1
		Mg/Ca	Mg/K	Ca/K	
Sand cultures supplied with a solution having a concentration of 1.75 atm.	R2 C7	0.27L	0.69	2.52H	2.81
	R2 C5	1.15	2.08	1.80	2.67
	R4 C2	3.85	1.39	0.36	2.50
	R3 C2	4.81	2.32	0.48	2.50
	R3 C1	11.53H	2.73H	0.24	2.38
	R3 C5	0.77	0.93	1.20	2.35
	R8 C1	1.92	0.18L	0.09L	2.25
	R2 C4	1.92	2.77	1.44	2.24
	R3 C4	1.44	1.39	0.96	2.23
Range		11.26	2.60	2.43	
Solution cultures of sub-optimal concentration 0.1 atm. (Shive)	R3 C1	11.55H	2.78H	0.24	1.39
	R7 C1	3.85	0.40	0.10	1.36
	R8 C1	1.92	0.18L	0.09L	1.35
	R4 C1	9.61	1.74	0.18	1.31
	R6 C1	5.77	0.69	0.12	1.31
	R5 C1	7.70	1.11	0.14	1.30
	R7 C2	0.96L	0.20	0.20	1.28
	R4 C2	3.85	1.39	0.36	1.22
	R3 C2	4.81	2.32	0.48H	1.19
Range		10.59	2.60	0.39	
Solution cultures of optimal concentration 1.75 atm. (Shive)	R1 C2	6.74	9.72H	1.44	1.11
	R4 C2	3.85	1.39	0.36	1.10
	R5 C2	2.88	0.83	0.29	1.08
	R3 C2	4.81	2.32	0.48	1.07
	R1 C4	2.40L	6.95	2.88H	1.07
	R5 C1	7.70H	1.11	0.14	1.07
	R3 C3	2.56	1.85	0.72	1.07
	R6 C1	5.77	0.69L	0.12L	1.06
	R2 C2	5.77	4.17	0.72	1.05
Range		5.30	9.03	2.76	

show a substantial agreement with those of the solution culture series of sub-optimal (0.1 atm.) concentration with respect to the range in the Mg/Ca and Mg/K ratios, and a close agreement with the optimal solution (1.75 atm.) series with respect to the range in the Ca/K ratios. As was the case when groups of best cultures of the three series were compared on the basis of dry weight of tops, there is a much closer agreement with respect to the range in value of the Mg/Ca ratio than for the range

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of Mg/K and Ca/K ratios. It appears, further, that the contact of the solution with the sand has not markedly changed the correspondence between the range of the Mg/Ca ratio and yield of tops and of roots.

Attention is called to the fact that the groups of best cultures on the basis of dry weight of tops were characterized throughout the three series by a comparatively narrow range in the value of the Mg/Ca ratio, while

TABLE XII

RANGE OF CATION RATIO VALUES AND RELATIVE DRY WEIGHTS OF ROOTS FOR THE POOREST NINE CULTURES OF WHEAT GROWN IN SAND AND IN SOLUTION CULTURES

	Culture Numbers	Cation Ratio Values			Yield of Roots Relative to that of Culture R1 C1
		Mg/Ca	Mg/K	Ca/K	
Sand cultures supplied with a solution having a concentration of 1.75 atm.	R1 C1	15.40H	11.10H	0.72	1.00
	R6 C1	0.48L	0.28L	0.58	1.30
	R5 C4	5.77	0.69	0.12L	1.31
	R1 C3	3.85	8.34	2.16	1.41
	R6 C2	1.92	0.46	0.24	1.46
	R5 C2	2.88	0.83	0.29	1.50
	R5 C3	1.28	0.56	0.43	1.51
	R2 C1	13.46	4.86	0.36	1.55
	R1 C4	2.40	6.94	2.88H	1.63
Range		14.92	10.82	2.76	
Solution cultures of sub-optimal concentration 0.1 atm. (Shive)	R5 C4	0.48	0.28L	0.58L	0.72
	R2 C6	0.64	1.29	2.16	0.76
	R2 C5	1.15	2.08	1.80	0.77
	R1 C7	0.55	2.78	5.04	0.78
	R2 C7	0.27	0.69	2.52	0.80
	R1 C8	0.24L	1.39	5.76H	0.80
	R3 C5	0.77	0.93	1.20	0.82
	R1 C5	1.54H	5.55H	3.60	0.86
	R4 C5	0.38	0.35	0.90	0.89
Range		1.30	5.27	5.18	
Solution cultures of optimal concentration 1.75 atm. (Shive)	R6 C3	0.64	0.23L	0.36L	0.87
	R2 C5	1.15	2.08	1.80H	0.90
	R4 C3	1.92	1.04	0.54	0.91
	R4 C4	0.96	0.69	0.72	0.91
	R5 C3	1.28	0.56	0.43	0.91
	R6 C2	1.92	0.46	0.24	0.91
	R3 C5	0.77	0.93	1.20	0.92
	R3 C6	0.32L	0.46	1.44	0.93
	R2 C3	3.21H	3.47H	1.08	0.93
Range		2.89	3.24	1.44	

the groups of best cultures on the dry weight of *roots* are characterized by a wide range in the same ratio.

Dry weight of roots. Range of cation ratios for the poorest nine cultures. Table XII shows the cation ratio values and their range for each series, the arrangement of the data being the same as that of the three tables directly preceding. The group of sand cultures shows a very wide

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range in the Ca/K ratios. The individual culture showing the poorest growth is characterized by a very high (15.40) Mg/Ca ratio, a very high (11.10) Mg/K ratio, and a low (0.72) Ca/K ratio value. A comparison of the sand and the solution cultures fails to bring out any correlation with respect to the ranges in the cation ratio values as set forth in this table.

V. Water-Requirements

(a) Transpiration Data

Throughout the entire growth period of these cultures the pots were weighed, and the transpiration loss was recorded, at the end of each 3-day interval. The total water loss for each culture was then determined by summing the losses thus recorded for the entire period. The transpiration data for the entire series has been presented in Table VI in connection with the dry weights of tops and roots. In that table the water-losses have been expressed in terms of the loss from culture R1 C1. To bring out the close agreement between the relative water-loss and dry weight of tops, these data have been plotted as shown in the graphs presented in

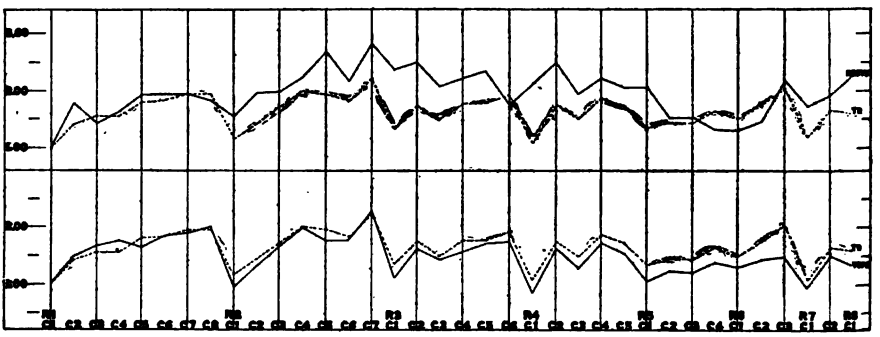

Fig. 6.—Relative transpiration and dry weight of tops and of roots of wheat grown in sand cultures for a period of 24 days.

figure 6. The abcissas are taken to represent the different cultures and the ordinates to indicate the relative dry weights and the transpiration losses relative to the loss from R1 C1 taken as unity. The broken line represents the variation in the relative dry weights of the individual cultures, while the solid line shows the variation in the water-loss from the same cultures.

The data presented in Table VI, and shown by the graphs of figure 6, appear to support the conclusion of Whitney and Cameron (44) and of other workers (20, 22) in the United States Bureau of Soils, to the effect that the total transpirational loss from a plant culture is approximately proportional to the growth made by the plants during the period of time considered. However, as has been pointed out by Livingston (19), this

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generalization may be considered as approximately true only for plants of the same species and of the same age, grown with the same aerial environment but in different solutions. This writer concludes that, under the conditions of his experiments the amount of water lost by transpiration is roughly proportional to the extent of the leaf surface of the plant, which, in turn, is related to the size and, therefore, roughly, to the dry weight. Shive (38, p. 375) has emphasized the point that this relation can hold only where the transpiration loss is determined almost entirely by the size of the plant (area of its surface), and not by internal physiological conditions.

(b) Water-Requirement per Gram of Dry Tops

Introductory considerations. The ratio between the amount of water lost by transpiration during a period, and the dry weight of plants produced in the same time, is a convenient term by which to express the water requirements of the plants, since such a ratio is the quantitative expression of the number of grams of transpirational water required to produce a single gram of dry substance.

An excellent review of the early literature upon this subject has been published recently by the United States Department of Agriculture (5) and need not be reviewed in this paper. However, attention should be called to the fact that the results obtained by Sorauer and by Heinrich in controlled solution cultures are in agreement with the recent work of Shive (38, p. 378) with respect to the effect of the total concentration upon the water-requirements of the plants. The results brought forward by these investigators appear to lead to the general conclusion that the higher the total concentration of the nutrient medium, the lower is the ratio between the amount of water transpired and the dry yield of the plants. However, this generalization can hold only within a certain range of concentrations, since it is obvious that as the concentration is increased a point must be reached finally where no growth is possible. The present sand culture series offers an opportunity to study the variation in the water-requirements of these wheat plants when grown in solutions of the same total concentration (approximately optimal), but with a wide variation in the relative partial concentrations of the three salts employed.

Presentation of data. In columns 2, 3 and 4 of Table XIII the absolute transpiration ratios for tops, for roots, and for the entire plant, including both tops and roots, are given. These values were obtained by dividing the total water-loss from the individual cultures by the corresponding dry weight yields. In the last column of the table are also presented the ratios of top yield to root yield.

The data of Table XIII are shown graphically in figure 7, in which the upper graph represents the variation among the individual cultures

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in the ratio of dry weight of tops to dry weight of roots, while the lower graphs represent the corresponding variations in the water-requirements for roots, for tops, and for entire plants including both tops and roots.

TABLE XIII

WATER-REQUIREMENTS FOR TOPS, ROOTS, AND ENTIRE PLANTS, AND THE RATIO OF TOPS TO ROOTS: WHEAT GROWN FOR 24 DAYS IN SAND CULTURES AND SUPPLIED WITH A THREE-SALT SOLUTION HAVING A TOTAL OSMOTIC CONCENTRATION OF 1.75 ATMOSPHERES

Culture Numbers	Water-Requirements[1]			Ratio[2] of Tops to Roots
	Tops	Roots	Entire Plant	
R1 C1	237L	1486	231	5.44
C2	258L	1160	211	4.49
C3	255L	1635	220	6.41
C4	240L	1401	205	5.82
C5	298	1475	247	4.89
C6	271L	1480	229	5.47
C7	280L	1472	235	5.25
C8	266L	1568	227	5.91
R2 C1	327H	1116	253	3.41
C2	293	1163	234	3.97
C3	291	1375	240	4.73
C4	277L	1334	229	4.82
C5	297	1072	233	3.61
C6	282	1239	230	4.40
C7	267L	1173	217	4.40
R3 C1	334H	828	238	2.50
C2	295	1031	230	3.94
C3	291	1068	228	3.67
C4	308	1181	244	3.84
C5	291	1130	232	3.95
C6	306	1618	258	5.28
R4 C1	370H	760	249	2.05
C2	298	1038	232	3.48
C3	326H	1125	245	3.59
C4	297	1247	240	4.20
C5	307	1240	246	4.04
R5 C1	348H	976	256	2.80
C2	323	1408	262	4.36
C3	324	1403	263	4.33
C4	325	1868	277	5.75
R6 C1	308	1711	261	5.56
C2	334H	1773	282	5.29
C3	378H	1380	297	3.64
R7 C1	332H	1021	250	3.07
C2	301	1266	243	4.21
R8 C1	328H	1040	249	3.14

[1] The water-requirement is determined by dividing the total water loss in grams by the dry weight value in grams.

[2] Obtained by dividing the dry weight of tops by the dry weight of roots.

The mean of the values for the water-requirements for tops is 307. It will be observed, in general, that the graph representing the ratio for tops (fig. 7) remains below the mean for the first twenty cultures, and that from this point forward the values equal or exceed the mean. From a study of the location of the cultures on the triangular diagram (fig. 8) it appears that a low water-requirement is associated with a low partial

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osmotic concentration of mono-potassium phosphate. The location of each of the areas of high and of low water-requirements for tops is shown in figure 8, in which the area of high values is indicated by crosses and the area of low values is marked by small circles. As in case of dry weights, the shaded areas include the nine cultures showing the highest values and an equal number of cultures showing the lowest values. In Table XIII the nine cultures showing the highest water-requirements are marked H, while the nine showing the lowest water-requirements are marked L. The numerical values of the ratios for the different cultures are given on the diagram. A study of figure 9 with respect to water-

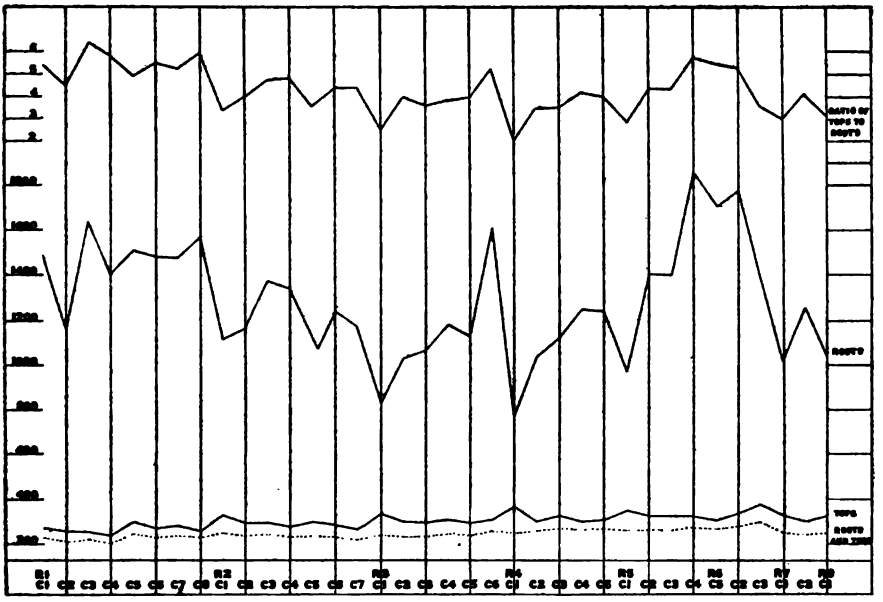

Fig. 7.—The water-requirement for the entire plant, for dry weight of tops and for dry weight of roots. The upper graph represents the variation in the ratios of tops to roots for the individual cultures.

requirements of the nine cultures giving the highest yield of tops and the nine cultures giving the lowest yields brings out the following facts. The average water-loss from the best nine cultures is 97 per cent greater than the water-loss from culture R1 C1, while the average yield of tops is only 89 per cent greater than that from culture R1 C1. The average water-loss from the poorest nine cultures is 27 per cent greater than that from culture R1 C1, while the average yield is 8 per cent greater than that from culture R1 C1. These observations are in accord with what might be expected from *a priori* grounds and from previous studies of water-requirement, namely, that favorable conditions for plant growth are generally associated with low water-requirements and that unfavorable conditions are concomitant with an increased water-requirement.

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(c) Water-Requirement per gram of Dry Roots

The mean of the values for the water-requirements for dry weight of roots is 1285. It will be observed from the graph for this value in figure 7 that there is a region of high values near each end, with a distinct region of low values between. From a comparison of this graph with the graph representing the ratio of top yields to root yields it will be seen that the two lines follow each other quite closely. In every instance a

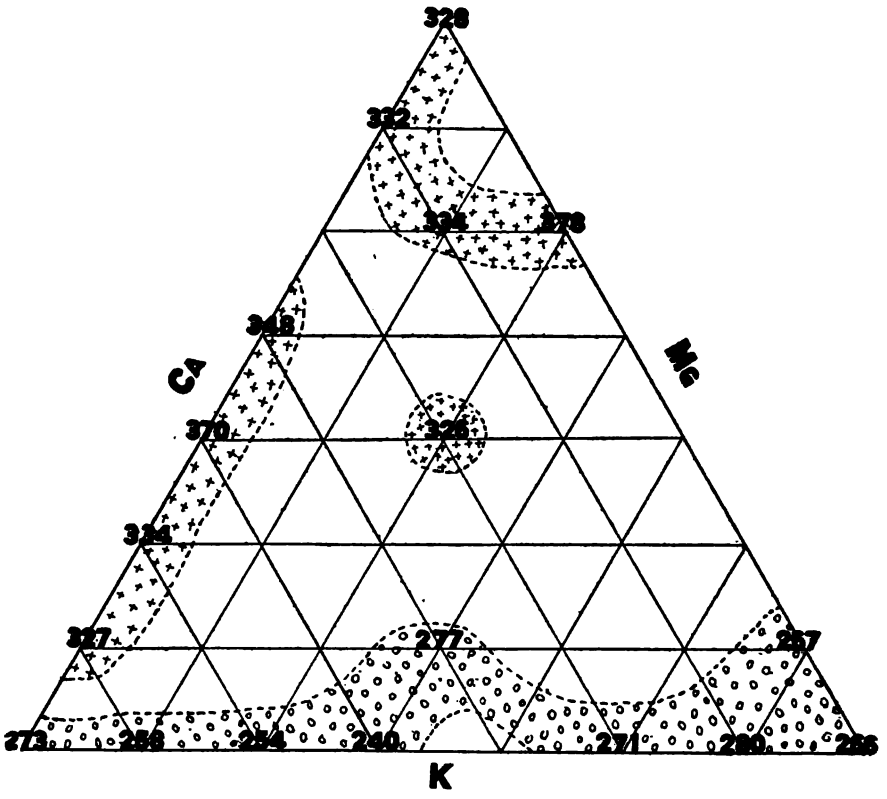

Fig. 8.—Areas of high and of low water-requirement values. The figures give the absolute values for 18 selected cultures.

high water-requirement corresponds to a high ratio of tops to roots and a low water-requirement corresponds to a low ratio of tops to roots. This is in harmony with what might be expected, since a high ratio of tops to roots suggests a large evaporating surface and a correspondingly high water-loss. As might be expected, the variation in the water-requirements is much greater for roots than for tops, the range for roots being from a minimum value of 760 (culture R1 C1) to a maximum value of 1868 (culture R5 C4).

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(d) Water-Requirement per gram of Entire Plant

In figure 7 the graph representing the water-requirement for the entire plant very closely parallels the graph giving the ratios for dry weight of tops, and both show the same tendency to rise as we proceed from left to right. However, there are absent from the former the very high points that are characteristic of the graph representing the water-requirement for dry weight of tops, and that characterize those cultures having low ratios of tops to roots.

From an inspection of the graphs presented in figure 7 it is apparent that for this series of sand cultures the range of the water-requirement values, between the different cultures throughout the series, is comparatively narrow, when the dry weight of the plant is taken as the basis from which this ratio is derived. The range for the entire series of 36 cultures is from a minimum of 205 (culture R1 C4) to a maximum of 297 (culture R6 C3).

VI. The effect of the Calcium-Magnesium Ratio upon the Growth Rate

(a) Introduction

As the result of his investigations concerning the physiological requirements of plants, Loew (23) has worked out an hypothesis concerning the functions of lime and magnesia in the nutrition and the growth of plants. Early in these studies he became greatly impressed by the reversal of the quantitative relations of the lime and magnesia in the straw and in the seeds of plants. A chemical study of the ash of many common field plants brought out the fact that in the grain the magnesia is greatly in excess of the lime, while in the straw the lime is in excess of the magnesia. For example, in case of wheat, the ash of the grain contains 3.5 per cent of CaO and 13.2 per cent of MgO, while the straw has 5.8 per cent of CaO and only 2.5 per cent of MgO (39). His study of these data and the results of his own investigations led him to formulate the theory concerning the relationship of calcium to the nucleo-proteids and the specific function of magnesium with respect to the translocation of phosphorus in the plant. The outstanding points of Loew's lime-magnesia ratio hypothesis are as follows:

(1) When lime occurs in the growth medium greatly in excess of magnesia, or when magnesia occurs greatly in excess of lime, an injurious effect upon the plant is to be noted.

(2) Either of these elements tends to neutralize the harmful effects of the other.

The outstanding conclusions which Loew has drawn from his own and other experimental data are as follows:

(1) Different plants require, for their best growth, slightly different proportions of lime and magnesia.[1]

[1] In practically all of the literature the results are discussed in terms of the ratio of CaO to MgO rather than the ratio of CaO to Mg, hence the use of the terms "lime" and "magnesia" in this paper.

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(2) The optimum ratio of lime to magnesia for oats is 1:1, for barley 2:1, and for buckwheat 1:3.

Upon the basis of various physiological investigations the writer just mentioned recommended that the amount of available lime and magnesia should be determined in agricultural soils in order to ascertain the proper lime-magnesia ratio. In a later publication Loew (24) discusses the physiological functions of calcium and magnesium in the plant. According to this writer, the presence of lime is necessary for the formation of certain calcium compounds required in the nuclei and in the chlorophyll bodies of the plant, and that magnesia assists in the assimilation of phosphorus, since magnesium phosphate can give up its phosphoric acid more easily than any other phosphate that occurs in the plant sap. The element calcium, therefore, is fixed in the organized structure of the cells, while magnesium is movable and serves as a carrier of assimilable phosphoric acid, which rôle can be repeated several times. But, if lime is taken up in excess, the assimilation of phosphoric acid will be made more difficult, since the acid will combine with the lime and thus diminish the chances for the formation of magnesium phosphate. As a result of the excess of lime, therefore, the amount of available phosphoric acid may be reduced and the plant may experience a partial starvation. If, on the other hand, magnesia is in considerable excess, the calcium of the nuclei and chlorophyll bodies may be transformed in the presence of the soluble magnesium salts into magnesium compounds, by an exchange of bases. This transformation of the calcium nucleo-proteids changes their capacity for imbibition, much to the detriment of the plant. According to the law of mass action, however, this transformation may be prevented by the simultaneous presence of dissolved calcium salts.

Since the publication of the lime-magnesia hypothesis of Loew, many other investigators have published experimental results bearing on the question here brought up. In a recent paper, Lipman (17) gives a critical review of 64 papers, all of which report results bearing upon the hypothesis of the lime-magnesia ratio. Some of these results appear to support Loew's hypothesis, others are inconclusive, and a third group seem to cast very serious doubt upon the necessity for definite ratios of lime to magnesia for the best growth of plants. Of the 64 papers considered by Lipman, 24 claim to give positive evidence of the need for a definite lime-magnesia ratio in the soil; 26 report negative results; and the remainder make no claim to either positive or negative results. In much of this literature the experimental evidence is not conclusive nor convincing, since the results obtained may be explained without resort to the hypothesis of Loew. To quote from Lipman: "The toxic effects of magnesium in excess or when applied to the soil when not in excess are easily explained on the basis of its own specific physiological properties

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and need no introduction of ratio considerations between itself and calcium to explain them. . . . . Calcium and magnesium are, of course, essential elements in the growth of plants. Their somewhat similar chemical nature does not give us leave, however, to place them in a similar physiological category, and indeed numerous investigations point to their total dissimilarity, so far as that is concerned; Loew's own investigations being perhaps the most important in support of that idea. But, in spite of that, it is inconceivable to any one who has in view the modern developments of plant physiology and physical chemistry, as well as the modern views on the soil and its solution, why it should be any more assumed that a proper ratio between calcium and magnesium is necessary than that a proper ratio between calcium and potassium, and between calcium and iron, and between calcium and other essential elements in the growth of plants, are necessary. . . . . The balance of the effects which are not accounted for by antagonism between the ions within the soil solution itself, may, so far as soils themselves are concerned, be just as easily explained on the basis of the effects of the applications on the physical conditions of the soil, on the chemical reactions following in the soil, on the bacterial flora, on the protozoan fauna, and on other fauna within the soil, as it can be, by introducing the rather far-fetched notion of the necessity of the lime-magnesia ratio."

It requires but a superficial examination of the literature to reveal the fact that, in a majority of the experiments heretofore carried out in this connection, no attempt has been made to differentiate between the purely physiological action of calcium and magnesium and what may be called their *environmental* effect. It has been found that, in certain soils where magnesia is in excess of lime, application of the latter substance has been very beneficial to the growth of crop plants. In such cases it is not necessary to suppose that the benefit comes from the correction of an unfavorable ratio between the calcium and magnesium. Indeed, it would seem just as plausible to assume that the good effect obtained is the result of the beneficial effect of the lime upon the bacterial flora of the soil, which effect may be so great as to obscure any physiological effects due to an improvement of the calcium-magnesium ratio, if such effects are actually present. In such a case, the addition of lime to the soil would be considered as affecting the bacterial environment of the plant, without reference to any direct effects produced upon the plant by the higher soil content of calcium.

The sand-culture method described in this paper seems to afford favorable conditions for studying the relation of the calcium-magnesium ratio to the growth of plants. The substratum of pure sand, in which the wheat plants of these studies were grown, furnishes physical conditions similar to those found in the open soil, but avoids many of the biological complications encountered with natural soils, which complications may

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very readily obscure the physiological relations between the plant and the salts of its surrounding medium. Furthermore, the renewal of the nutrient solutions at the end of every 3-day period serves to maintain within the medium a fairly constant condition with respect to all of the salts contained in the solutions. If a definite value of the calcium-magnesium ratio is essential for the best development of the seedling, evidence of that fact should be expected from such a complete series of sand cultures as the one here employed, since one of the cation ratios (Mg/Ca) is very closely related to the much discussed lime-magnesia ratio. If, on the other hand, the physiological processes of the plant are dependent in this connection, upon the proper balancing of the solution as a whole, we should not expect to find here any definite correlation between the value of the calcium-magnesium ratio and the growth and development of the seedlings.

(b) Discussion of Data

Examination of the data presented by the diagram of figure 2, with respect to the osmotic proportions of calcium nitrate to magnesium sulphate in a number of selected cultures, brings out some points in this connection. In the culture giving the best growth of tops (R2 C7) the osmotic ratio of $Ca(NO_3)_2$ to $MgSO_4$ is 7:1, while in the culture giving the poorest growth of tops (R4 C1) this ratio value is 1:5 (C.2:1.0). The average ratio of $Ca(NO_3)_2$ to $MgSO_4$ for the best nine cultures has a value of 2.4:1, while for the poorest nine cultures this value is 1:2.9. It is thus evident that good growth is associated with a high osmotic ratio of $Ca(NO_3)_2$ to $MgSO_4$ and that the poor cultures are characterized by a low value of this ratio. We are not justified, however, in drawing from these results, any definite conclusions with respect to the effect of the calcium-magnesium, as such, because of the fact that much of the superior growth, in the culture where $Ca(NO_3)_2$ is in excess, may be ascribed to the presence of a large amount of the NO_3 radical, which is known to be favorable to very vigorous vegetative growth.

It has long been known that most higher plants absorb the greater part of their nitrogenous food material in the nitrate form. Hellriegel and Wilfarth's (14) experiments with barley in sand cultures, where the nitrogen was supplied in the form of $Ca(NO_3)_2$, show a very marked increase in the amount of dry matter produced, as the amount of nitrogen is increased up to a certain maximum, above which additional increments of $Ca(NO_3)_2$ produce no further effect. It has been suggested by Russell (31) that the increasing effects produced by successive increments of nitrogen up to this maximum may be due to the fact that the additional nitrate not only increases the concentration of the radical in the solution, but that it also increases the extent of the absorbing surface of the roots and also that of the leaves. The process thus seems to bear some re-

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semblance to autocatalysis, in which one of the products of the reaction serves as a catalyzer and hastens the reaction. This process, however, cannot go on indefinitely, because the time must come when some limiting factor will intervene and prevent further increase. Von Seelhorst (35) studied the effect upon the oat plant of the addition of nitrogen to soils containing different amounts of water. The experiment included 9 pot-cultures, which were divided into 3 series of 3 cultures each. In one series the soil used was just moist; in the second series the moisture content was slightly higher; while in the third series the soil was kept very moist. One pot in each series received no nitrogen, a second received 0.5 gm. of $NaNO_3$, and the third received double that amount. In those cultures where only a small amount of moisture was present the addition of 0.5 gm. of nitrogen was without effect, the supply in the soil being sufficient for the needs of the plants, the water supply rather than the supply of nitrogen being the limiting condition. When more water was present the plants were able to make more growth and to utilize more nitrogen. The addition of one increment of nitrogen to the slightly moister soil of the second pot increased the produce by 10 gm., but the addition of the second increment was without additional effect, the water supply having again become the limiting condition. When a liberal amount of water was supplied, the first increment of nitrogen gave an increase of 20 gm., and the addition of another increment gave a still further increase of 15.5 gm.

In the present studies, where the moisture content of the soil was kept very nearly optimum, it might be expected that the dry weight of the plants would increase, with increase in the nitrogen content of the nutrient solution, until some condition other than water-supply became the condition limiting growth. Attention has been called to the fact that this series showed a gradual increase in the dry weight of tops in the cultures of row 1, as we proceed from C1, with 1 tenth of its total osmotic concentration due to $Ca(NO_3)_2$, to C8, with 8 tenths of its total concentration derived from that salt. As we pass upward toward the apex of the triangular diagram (fig. 2), however, the effect of additional increments of $Ca(NO_3)_2$ above the amount present in Culture C1 is not so apparent and soon entirely disappears except in the case of the first increment. The results obtained in this series suggest that, in the cultures represented as lying in the upper region of the diagram, the amount of KH_2PO_4 present in the solution may be the limiting condition, beyond the second culture from the left margin in each row.

The effect of the ratio of calcium nitrate to magnesium sulphate, upon the growth of tops, is brought out by an inspection of the cultures represented as occupying row 1, at the base of the triangular diagram (fig. 1). All of the cultures in this row have the same partial osmotic concentration of KH_2SO_4; namely, 1 tenth of the total osmotic concentration.

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Passing to the right from culture R1 C1, the osmotic concentration of $Ca(NO_3)_2$ increases by increments of 1 tenth, up to a partial concentration of 8 tenths of the total, culture R1 C8. As the partial osmotic concentration of $Ca(NO_3)_2$ *increases* from left to right, that of $MgSO_4$ *decreases* at the same rate. With the exception of one culture (R1 C5), the dry weight of tops increases with increasing partial osmotic concentration of $Ca(NO_3)_2$, and with decreasing partial concentration of $MgSO_4$. An inspection of harvest record (Table VI) shows that culture R1 C8, with the osmotic ratio, $Ca(NO_3)_2$ to $MgSO_4$, of 1:8 produced 100 per cent greater dry weight of tops than did culture R1 C1, where the value of the $Ca(NO_3)_2$—$MgSO_4$ ratio was 1:0.12. The greatest difference between individual cultures is found in the case of cultures R1 C1 and R1 C2, where a decrease of one increment in the partial osmotic concentration of $MgSO_4$ and a corresponding increase in the partial osmotic concentration of $Ca(NO_3)_2$ resulted in an increase of 48 per cent in the yield of tops. Increases of a similar magnitude are found when cultures R2 C2, R3 C2, and R4 C2, are each compared with the first culture in its row.

Considering the culture occupying the right margin of the diagram (fig. 3, A), all of which had the same proportion of $MgSO_4$, high yields of tops are associated with large osmotic partial concentrations of $CaNO_3$ and with low concentrations of KH_2PO_4. Proceeding toward the upper apex of the triangle, by the second row, the dry weights decrease with decrease in the value of the ratio, $Ca(NO_3)_2$ to $MgSO_4$. Osterhout (28) has pointed out that potassium may inhibit more or less completely the poisonous effect of excessive quantities of magnesium. Working with a marine alga (*Enteromorpha Hopkirkii*), he found these plants lived 5 times as long in a mixture of magnesium and potassium chlorides as in pure magnesium chloride, and 3 times as long as in pure potassium chloride. This same writer also grew wheat and other flowering plants in mixtures and in pure solutions of the same salts, with similar results. During a period of 40 days the wheat roots made a growth of 10 mm. in a .0937 m. $MgCl_2$ solution, but in a mixed solution (of the same total concentration) of KCl and $MgCl_2$ the growth was 153 mm. for the same time period. The results of the present investigation indicate that KH_2PO_4 is not effective in balancing the nutritive solution after a certain minimum ratio of calcium nitrate to magnesium sulphate has been reached. In this instance a marked decline in yield is shown between R2 C7 and R3 C6 as we go from a calcium nitrate to magnesium sulphate ratio of 7:1 in the former to a ratio of 6:1 in the latter.

It is obvious that if there is an optimum calcium-magnesium ratio value for the best growth of plants it can hold only within certain limits. For example, if the total concentration of the nutrient solution is too high, plants will fail to make satisfactory growth, whatever the

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value of the calcium-magnesium ratio may be. Furthermore, the omission of KH_2PO_4 from the solution, or an addition of an excess of this salt, would make impossible the growth of plants in such a medium, regardless of any consideration of the calcium magnesium relation.

LITERATURE CITED

(1) BREAZEALE, J. F.
1905. Effect of the concentration of the nutrient solution upon wheat cultures. *In* Science, v. 22, p. 146-149.

(2) BREAZEALE, J. F.
1906. Effect of certain solids upon the growth of seedlings in water cultures. *In* Bot. Gaz., v. 41, p. 54-63.

(3) BRIGGS, J. L.
1905. On the adsorption of water vapor and of certain salts in aqueous solutions by quartz. *In* Jour. Phys. Chem., v. 9, p. 617-640.

(4) BRIGGS, J. L.
1912. The wilting coefficient for different plants and its indirect determination. *In* U. S. Dept. Agr. Bur. Plant Indus. Bul. 230, p. 13.

(5) BRIGGS, J. L., and SHANTZ, H. L.
1913. The water requirements of plants. II. A review of the literature. U. S. Dept. Agr. Bur. Plant Indus. Bul. 285.

(6) CAMERON, F. C., and BELL, J. M.
1905. The mineral constituents of the soil solution. U. S. Dept. Agr. Bur. Soils Bul. 30, p. 42-62.

(7) CZAPEK, F.
1913. Biochemie der Pflanzen. Jena.

(8) DANDENO, J. B.
1904. The relation of mass action and physical affinity to toxicity. *In* Amer. Jour. Sci., s. 4, v. 17, no. 102, p. 437-458.

(9) DUGGAR, B. M.
1911. Physiology of Plant Production. New York.

(10) FINDLEY, ALEXANDER.
1913. Osmotic Pressure. London.

(11) FRANK, A.
1866. Ueber die Bedeutung des Kochsalzes fur die D· ıgung des Untergrundes. *In* Landw. Vers. Stat., Bd. 8, p. 45.

(12) GORE, G.
1894. Decomposition of liquids by contact with powdered silica, etc. *In* Chem. News, v. 69, p. 23, 33, 34.

(13) HARRIS, F. S.
1915. Effect of alkali salts in soils on the germination and growth of tops. *In* Jour. Agr. Research, v. 5, no. 1, p. 12-27.

(14) HELLRIEGEL, H., and WILFARTH, H.
1888. Untersuchungen über die Stickstoffnahrung der Gramineen und Leguminosen. *In* Ztschr. Ver. Deut. Rubenzuker Indus. (Quoted from Russell, E. J. [Ref. No. 31], p. 31).

(15) JENSEN, G. H.
1907. Toxic limits and stimulation effects of some soils and poisons on wheat. *In* Bot. Gaz., v. 43, p. 11-14.

(16) KNOP, W.
1859. Ein Vegetationversuch. *In* Landw. Vers. Stat., Bd. 1, p. 181-202.

Digitized by Google

(17) LIPMAN, C. B.
1916. A critique of the hypothesis of the lime-magnesia ratio. *In* Plant World, v. 19, p. 83-105, 119-135.
(18) LIVINGSTON, B. E.
1903. The Rôle of Diffusion and Osmotic Pressure in Plants. Chicago.
(19) LIVINGSTON, B. E.
1905. Relation of transpiration to growth in wheat. *In* Bot. Gaz., v. 40, p. 178-195.
(20) LIVINGSTON, B. E.
1907. Further studies on the properties of unproductive soils. U. S. Dept. Agr. Bur. Soils Bul. 36.
(21) LIVINGSTON, B. E.
1915. Atmometers and porous cup atmometry. *In* Plant World, v. 18, p. 21-30, 51-74, 95-111, 143-149.
(22) LIVINGSTON, B. E., BRITTON, J. C., and REID, F. R.
1905. Studies on the properties of unproductive soils. U. S. Dept. Agr. Bur. Soils Bul. 28.
(23) LOEW, O.
1892. Ueber die physiologischen Functionen der calcium und magnesium salze im Pflanzenorganisms. *In* Flora., Bd. 75, p. 368.
(24) LOEW, O., and MAY, D. W.
1901. The relation of lime and magnesia to plant growth. *In* U. S. Dept. Agr. Bur. Plant Indus. Bul. 1, p. 16-17.
(25) MCCALL, A. G.
1915. A new method for the study of plant nutrients in sand cultures. *In* Jour. Amer. Soc. Agron., v. 7, no. 5, p. 250-252.
(26) MCCALL, A. G.
1916. The adsorption of potassium by the soil. *In* Jour. Phys. Chem., v. 20, no. 1, p. 51-63.
(27) NÄGELI, C. VON,
1893. Ueber oligoddynamische Erscheinungen in lebenden Zellen. *In* Denkschr. Schweiz. Naturf. Gesell., Bd. 33, p. 1-21.
(28) OESTERHOUT, W. J. V.
1908. The antagonostic action of magnesium and potassium. *In* Bot. Gaz., v. 45, p. 117-124.
(29) PARKER, E. G.
1913. Selective adsorption by soils. *In* Jour. Agr. Research, v. 1, no. 3, p. 179-188.
(30) PFEFFER, W.
1900. The Physiology of Plants. Translated by A. J. Ewart, Oxford.
(31) RUSSELL, E. J.
1912. Soil Conditions and Plant Growth. London.
(32) SACHS, J.
1859. Ueber den Einfluss der chemischen und physikalischen Beschaffenheit des Bodens auf die Transpiration der Pflanzen. *In* Landw. Vers. Stat., Bd. 1, p. 203-240.
(33) SCHREINER, O., and SKINNER, J. J.
1910. Ratio of phosphate, nitrate and potassium on adsorption and growth. *In* Bot. Gaz., v. 50, p. 1-30.
(34) SCHREINER, O., and SKINNER, J. J.
1910. Some effects of a harmful organic soil constituent. *In* U. S. Dept. Agr. Bur. Soils Bul. 70.

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(35) SEELHORST, C. VON,
1899. Ueber den Wasserverbrauch der Haferpflanze bei verschiedenem Wassergehalt und bei verschiedener Dungung des Bodens. *In* Jour. Landw., Bd. 57, p. 369-378.
(36) SHIVE, J. W.
1914. The freezing points of Tottingham's nutrient solutions. *In* Plant World, v. 17, p. 345-353.
(37) SHIVE, J. W.
1915. A three-salt nutrient solution for plants. *In* Amer. Jour. Bot., v. 2, p. 157-160.
(38) SHIVE, J. W.
1915. Physiological balance in nutrient media. *In* Physiol. Research, v. 1, p. 327-397.
(39) SNYDER, HARRY.
1905. The Chemistry of Plant and Animal Life, p. 173. New York.
(40) TOTTINGHAM, W. E.
1914. A quantitative chemical and physiological study of nutrient solutions for plant cultures. *In* Physiol. Research, v. 1, p. 133-245.
(41) TRUE, R. H., and OGLEVEE, C. E.
1904. The effect of the presence of insoluble substances on the toxic action of poisons. *In* Science, v. 19, p. 421-424.
(42) TRUE, R. H., and OGLEVEE, C. E.
1905. Idem. *In* Bot. Gaz., v. 39, p. 1-21.
(43) WASHBURN, E. W.
1915. Principles of Physical Chemistry, p. 158-164. New York.
(44) WHITNEY, M., and CAMERON, F. K.
1904. Investigations in soil fertility. U. S. Dept. Agr. Bur. Soils Bul. 23.
(45) WILLIAMS, A. M.
1914. "Negative" adsorption. Trans. Faraday Soc., v. 10, p. 155.
(46) WOODWARD, J.
1699. Thoughts and experiments on vegetation. Phil. Trans. Roy. Soc. London, v. 21, p. 382-398.

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PLATE I.

Wheat cultures about 20 days old, showing form of pot and arrangement for renewing the solutions. Scale is inches.

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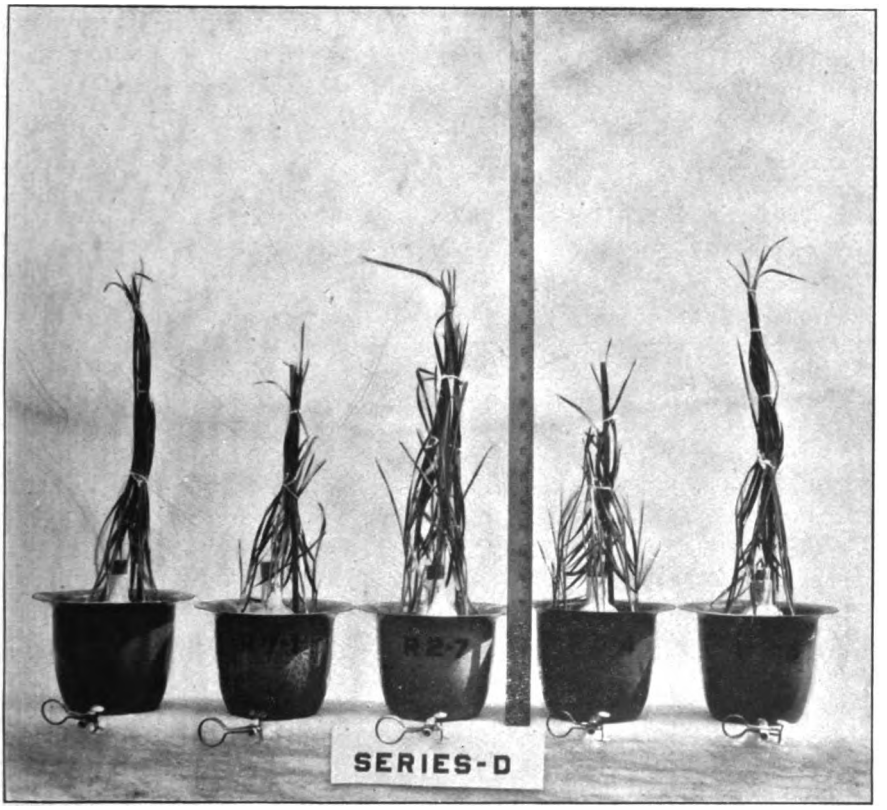

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SOME BACTERIOLOGICAL STUDIES ON AGAR AGAR[1]

By

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By virtue of the ability which very small quantities of agar agar have of forming with water a comparatively non-nutrient gel, it was early used by Robert Koch in 1881, for the cultivation of certain pathogenic bacteria. Koch (22) who had long been in search of a transparent jelly, quickly recognized its value and brought it into general use a few years later. He used beef broth as the nutrient base, and with this medium cultivated a great many species of bacteria. It was he who first pointed out the great advantage of the use of solid media for the isolation and cultivation of bacteria, and with the bounteous harvest of original and important suggestions that was reaped from Koch's classical series of investigations, the new science of bacteriology attained unthought of importance, and at this period modern bacteriology may be justly said to have had its birth.

Miquel (29), in 1878-1885, had probably prior to Koch's work also used nutritive vegetable jellies in his researches on bacteria.

After the impetus given by these early workers, the use of agar as a base in solid culture media became firmly established, until to-day agar media are perhaps more used than any other. With the increase in popularity of this new substance, questions naturally arose concerning the proper methods of preparation, filtration, sterilization, adjusting the reaction and other points. Thus the period from 1882 to 1892 may be said to include problems of methodology principally. A great number of media were proposed, as well as formulae for preparation, and methods of sterilization, filtration, neutralization and the like. The subject of agar media was investigated in almost every laboratory. These investigations cannot be reviewed here as the number is too great, and besides, the greater number of the proposed methods and formulae have fallen into disuse, in the light of our modern knowledge on the subject. They are, however, of historical interest in that they show the vast amount of persevering and conscientious travail which must be encountered before any science can be said to rest on solid foundations.

Among the first to use peptone in agar media was Edington (11); Biondi (2) used the whites of eggs to clarify media in 1887. The cotton filter was probably introduced by Rosenbach (38) in 1884. Puccinelli

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(35), Mace (27), Schottelius (39), Richter (37) and many others have made notable contributions to the question of agar media. More recently Moore (30), Dominikiewicz (9) and Ravenel (36) have presented more precise and refined methods of preparing agar media. It may be said that almost every worker in the field of bacteriology employs his own peculiar mode of procedure and many times methods used successfully by one investigator fail utterly in the hands of another.

It is to be greatly deplored that such an entire lack of uniformity exists in the different steps in the preparation of the most commonly used culture media. The American Public Health Association (41) has already standardized the methods to be used in the bacteriological examination of water. Steps have also been taken to provide standard methods and media for all soil biological work, as soon as sufficient data are at hand to warrant definite conclusions.

The following data are presented in the hope that they will be of some value in the speedy attainment of these ends.

The chemical properties of agar and a proposed method of purification are discussed by the author in another paper (12). It is shown that agar agar contains considerable amounts of protein substances and other soluble nutrients. The question as to whether these nutrients are available for microörganisms is raised.

I

Can the Nutrients Contained in Agar Agar be Utilized by Microorganisms?

In the present experiment samples of ordinary commercial agar agar were washed with cold 0.5 per cent HCl solution (media usually contain more acid than this), for several hours, the filtrate evaporated to dryness and subsequently tested for the presence of Ca, Mg, S, N, Na and K. Abundant amounts of the first three elements were present, along with smaller amounts of Na and K. About 37 per cent of the nitrogen was found to be soluble. These results show that some of the nutrients present in agar agar are partly soluble in cold slightly acidulated water; probably more would become soluble in the relatively large quantity of water present in most media, if the acidity were increased in the process of sterilization at high temperatures. *Bacillus subtilis, Baccillus proteus* and several soil infusions on being inoculated into test tubes containing mother liquor of washed agar, which had been neutralized, thrived, a putrefactive odor of H_2S being given off from some of the tubes. This shows that the sulphur was used by the bacteria.

In order to ascertain whether the nitrogen present in agar agar is available for microörganisms, the agar agar itself was used as the source of nitrogen in an ammonification experiment.

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Series I

One hundred grams of quartz sand, 35 gm. of powdered agar, equivalent to 134 mg. of N and 108.6 gm. of H_2O (1½ optimum) were placed in 500-c.c. Erlenmeyer flasks, and each except the check inoculated with 2 c.c. of a 1:10 soil infusion. After thoroughly mixing the contents, the flasks were incubated at about 22° C. for 7 days, and the ammonia determined by distillation with heavy Mg 0.

Series II

Two hundred grams of quartz sand, 17.5 gm. of agar (67 mg. N.), 2 gm. of glucose, 60 c.c. of water (1½ optimum) were similarly inoculated with 2 c.c. of a 1:10 soil infusion, incubated and determined as in Series I. A check flask inoculated with 2 c.c of sterilized soil infusion was also treated and estimated in the same manner.

Series III

Three hundred c.c. of water, 5.833 gm. of agar agar, and 1 gm. of dextrose, in 500-c.c. flasks were sterilized at 15 pounds pressure for 15 minutes in an autoclave. After removing and cooling to 40° C. the flasks, except the check which was inoculated before sterilization, were inoculated with 5 c.c. of 1:10 soil infusion and incubated as before. The results shown in Table I were obtained.

TABLE I

AMMONIFICATION OF AGAR AGAR

Series	Mg. N. Added	Mg. N. Converted to NH_3	Per cent Conversion
I	134.00	31.32	23.40
	134.00	24.50	18.30
II	67.00	7.77	11.60
	67.00	15.82	23.60
III	21.46	—0.14	—0.65
	21.46	0.49	2.33

The results of Series I and II show that a part of the nitrogenous matter present in agar agar can be converted by microörganisms into ammonia. The odor of putrefaction was plainly noticeable when the plugs were removed from the flasks after incubation. The conditions under which Series III was allowed to proceed approximate closely those of ordinary agar media. In this case there is little if any ammonification, but when it is considered that any decomposition which takes place must be of an anaerobic nature, except directly at the surface of the flask, such results are to be expected. They show that little if any nitrogen is made use of by microörganisms under the conditions of the experiment.

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It is of passing interest at this point to merely mention the work of the Pringsheims (34). They showed that agar agar may be a source of energy in the assimilation of atmospheric nitrogen. In a 0.4 per cent dextrose and 0.2 per cent agar agar mixture, inoculated with *Bacillus gelaticus* and *Bacillus clostridium,* 26.6 mg. N. were assimilated per gram of agar agar; where .005 per cent $(NH_4)_3PO_4$ was substituted for the dextrose, the amount fixed was 15.4 mg. Similar results were obtained with *B. gelaticus* in association with *Azotobacter chroöcoccum.* These results show the origin of the nitrogen required by vegetable and animal organisms in the sea as both Azotobacter and Clostidria occur in sea water, and that the agar present in the red and brown algae is rendered available as a source of energy with the aid of *B. gelaticus.*

B. gelaticus is a deep sea organism capable of liquefying agar by means of the enzyme gelase. It is fully described by Gran (15).

To test out further the question of the availability of the salts present in agar, slants of non-nutrient agar, made up with distilled water, were inoculated with the following organisms: *Bacillus Rutida, Bacillus vulgatus, Bacillus megatherium, Bacillus subtilis, Bacillus butrycus, Bacillus mesentericus, Bacillus prodigiosus, Saccharomyces* (sp ?). *Penicillium viridicatum* Westling and *Trichoderma Koningi.* Examinations of the tubes after 26 days showed that all the organisms except, *B. butrycus, B. mesentericus* and possibly *B. vulgatus,* had grown to a greater or lesser extent. The yeast, the two fungi and *B. subtilis* grew noticeably better than the others, which fact would seem to indicate that the higher organisms are better able to extract food from agar than bacteria. The culture of *B. prodigiosus* which was used, formed a deep red color on ordinary media, but on non-nutrient agar no pigment at all was produced. This organism is especially susceptible to changes in environment. Luckhardt (26) shows its variability in pigment production on different media. When grown in an atmosphere of CO_2 it is colorless. Duggar (10) states cultures may lose their pigment when grown in acid media. It has been the author's observation that the pigment is much deeper on alkaline than on acid media.

The culture of *B. subtilis* was found on microscopic examination to consist entirely of spores. This observation is akin to that of Stephandis (42), who found that spore formation in *Bact. anthracis* increased as the media became poorer in nutrients.

This experiment indicates that although microörganisms do not thrive well on non-nutrient agar, yet they are able to grow and multiply on it to some extent.

Summarizing, the whole experiment shows that although there are considerable amounts of soluble nutrients including nitrogenous substances in agar, they are used by microörganisms under ordinary conditions, only

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to a very limited extent. If the conditions are made favorable however, microbial activity becomes fairly marked, one of the results being a transformation of a part of the protein into ammonia. It is advisable at any rate to use a high grade or purified agar in refined bacteriological work.

METHODS

Before passing on to the following experiments a brief resumé of the methods employed will be inserted here.

In general two media were used as follows:

I. Peptone—KCl Agar (Chamot)	
Agar	20 gm.
Peptone	20 gm.
KCl	6 gm.
Tap water	1000 c.c.
Acidity at 50° C.	1% HCl

II. Lipman & Brown's (25) Modified Synthetic Agar	
Agar	15 gm.
Peptone	.05 gm.
K_2HPO_4	0.5 gm.
$MgSO_4$	0.2 gm.
Dextrose	10 gm.
$Fe_2(SO_4)_3$	Trace
Distilled Water	1000 c.c.
Acidity unadjusted.	

Care was taken to prepare the media always in the same way so as to get as little variation as possible between different batches. Leitz's "shred agar" and "peptone" were used throughout the investigation. The agar was washed for several hours in about one-half the amount of water to be finally added to the medium. The water was usually changed several times. The finely cut, swollen shreds were separated from the water by filtration through cheesecloth, made up to weight with water, and the other ingredients added. Titrations were made at 50° C., as this temperature is nearer that of incubation, and because peptone media become more acid on heating. At first egg albumen was used to clarify the medium but this was afterwards deemed unnecessary, besides consuming considerable time. The tubed media were usually liquefied by means of flowing steam in an autoclave, after which the tubes were placed in a large water bath kept at 45° C. This procedure reduces to a minimum any variations in the temperature of the medium at the time of pouring the plates. Dilution water containing the bacteria was never allowed to remain exposed in the plates, for more than a few minutes. The period allowed for development of colonies of bacteria was 120 hours. Plates were poured in series of five, as in the author's opinion that number is desirable to obtain truly comparable data. In reporting the results, four plates are given, as it was found that that number usually check reasonably closely. For soils the usual dilutions used were 1:50,000 and 1:100,000. In the examination of soil, 20 gm. was placed in 200 c.c. of sterile tap water and shaken vigorously for five minutes. Dilutions made from this infusion were shaken for one minute each.

The soil and water samples were obtained on or near the College Farm, and were chosen so as to include as large a variety as possible.

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Soils

I. Penn sandy loam (acid).
II. Sassafras silt loam (slightly acid).
III. Penn loam (red shale) (neutral).
IV. Alloway clay (very acid).
V. Penn sandy loam (neutral).
VI. Sassafras gravelly loam (neutral).

Water Samples

I. Ground water (Campus spring).
II. Surface water (Campus pond).
III. Surface water (Reservoir).
IV. Ground water (shallow well).

II

Comparison of Counts on Ordinary and Purified Agar

The purified agar described in another paper (12), was compared with ordinary agar in media made up in the same way. The essential steps in the process of purification are first washing the crude agar agar with dilute acetic acid, and finally with water, then dissolving in hot water to make a 5 per cent solution. This is precipitated by an excess of alcohol or acetone, filtered, washed and dried.

TABLE II
COMPARISON OF "PURIFIED" WITH "ORDINARY" AGAR[1]

Title	Soil I		Soil IV	
	Purified Agar	Ordinary Agar	Purified Agar	Ordinary Agar
MEDIUM I				
Plate I	3.95	3.80	2.15	2.20
Plate II	4.10	4.00	2.50	2.25
Plate III	4.30	4.05	2.10	2.40
Plate IV	3.60	4.25	2.35	1.90
Average	3.99	4.02	2.27	2.19
MEDIUM II				
Plate I	6.40	6.75	2.65	3.00
Plate II	6.40	5.95	2.50	3.10
Plate III	5.80	6.20	3.15	2.60
Plate IV	6.15	6.50	3.00	3.30
Average	6.35	6.19	2.82	3.00

[1] Bacteria are expressed in millions per gram of soil.

The same weight of both purified and ordinary agar agar was used. This introduces a slight error due to the small increase in concentration in the case of the purified product. Two soils and two media were used in this experiment, the data of which are given in Table II.

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The data show that there is little if any difference between the media made up with ordinary and with purified agar agar. Perhaps there is a slight tendency in favor of the unpurified agar medium but this may be due to either the soluble nutrients in the agar or else to an antiseptic action caused by the presence of a small quantity of alcohol which may not have been removed in the process of washing. The purified agar media were much the clearer, thereby making the colonies more readily distinguishable and consequently easier to count.

Agar agar, purified in the manner suggested, has no appreciable effect on the bacterial counts from soils as compared with ordinary agar agar.

III

The Influence of Varying Concentrations of Agar agar in Culture Media on Bacterial Counts

Among the many unsolved problems appertaining to bacteriological methods and the preparation of culture media is that of the proper concentration of agar which will give the best results. At the present time all sorts of concentrations extending from 1 to 2.5 per cent are usually employed. It is the purpose of this work to throw some light on the influence of agar-concentration in media, on bacterial counts. Dr. E. M. Chamot of Cornell University first brought this question to the writer's attention, with regard to the sanitary examination of water.

Very little research has been done on the question of agar concentration in media. Hesse (17) showed that the size of the bacterial colonies developing on gelatin was not larger than those on 1 per cent agar. Gelatin plates reached their maximum development in 6 to 10 days, while it required 11 to 15 days for agar plates. A greater number of organisms grew on agar than on gelatin.

Hinterburg and Reitman (21) determined the effect of varying concentrations of agar on the growth of *Pseudomonas pyocyaneus*. A very slight change in concentration caused much change in the vitality and character of the growth of this organism.

Gazetti (14) has pointed out the marked effect of composition of media on the growth of chromogenic bacteria.

Heim (16), Moore (31), Fisher (13), Conn (6) and others have recommended about 12.5 gm. of agar per liter of medium. Lipman and Brown (25), Temple (44), Cook (7) and a host of other investigators have used a concentration of 1.5 per cent agar. The committee on Standard Methods for the Examination of Water and Sewage (41) report that 10 gm. of agar per liter of medium, appears to give higher and more consistent counts than the use of greater amounts.

Wolf (48) experimented on the growth of a number of bacteria on several media with varying concentrations. He states that most bacteria

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grow well at a concentration of 50 per cent dry matter in the nutrient medium, but the growth is poor in media containing 60 per cent dry matter. He makes the observation that the pigment forming bacteria appear to thrive best on the more concentrated media.

TABLE III

EFFECT OF CONCENTRATION OF AGAR ON BACTERIAL COUNTS[1]

MEDIUM I

in Media Per cent	0.5	1	1.5	2	2.5	3.0	4.0
Soil I	3.80	4.35	5.00	5.45	5.60	4.70	5.30
	3.20	4.20	5.30	5.10	4.80	5.60	4.60
	3.10	3.80	4.40	4.75	4.50	4.80	4.80
	3.63	4.70	4.55	5.20	4.70	5.05	5.25
Average	3.431	4.26	4.81	5.12	4.90	5.04	4.99
Soil II	3.90	7.80	6.75	7.75	6.85	6.70	6.60
	4.05	7.40	7.25	7.80	6.60	7.35	6.50
	4.50	7.50	7.35	7.10	7.05	6.75	6.90
	3.60	7.70	7.50	6.75	6.40	7.00	6.00
Average	4.01	7.60	7.21	7.35	6.73	6.94	6.50
Soil III	7.50	7.45	8.55	8.40	5.75	4.35	6.00
	6.50	8.30	8.50	8.50	5.10	6.10	6.50
	5.50	8.25	8.05	10.00	6.35	6.25	5.76
	5.75	8.55	8.35	8.00	6.50	6.40	5.50
Average	6.31	8.14	8.36	8.73	5.93	5.78	5.94
Soil IV	12.30	13.80	13.20	12.90	12.60	12.30	10.20
	10.80	13.20	13.80	12.60		12.30	10.50
	11.70	11.70	13.20	15.00	12.00	12.00	9.00
	9.90	12.60	15.60	16.50	12.30	10.20	9.90
					12.90		
Average	11.18	12.83	13.95	14.25	13.20	11.70	9.90

[1] Bacteria are expressed in millions per gram of soil. Dilutions 1:50,000.

Besides the researches just reviewed, there are innumerable recommendations from every side, as to the concentration of agar in media. All amounts from 4.5 gm. per liter to 40 gm. per liter appear to have been used, but no systematic researches have been discovered in the literature touching upon a comparison of different concentrations, with different media and a number of soils. Since agar agar always has a certain amount of moisture (about 16.5 per cent), it can be seen that unless it is dried an error will be introduced in weighing out the exact amount desired. But the moisture in agar agar samples from different sources is constant or nearly so (within 3 or 4 per cent at most); hence the error is not great. Plain washed agar was used in this experiment.

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The data in Tables III and IV, graphically represented in figure 1, give the results obtained in the course of this investigation.

Discussion of Results

An inspection of the data, curves and plates shows a gradual increase in numbers of bacteria from an agar concentration of 0.5 per cent to about 1.5 to 2.0 per cent where the maximum is reached. In concentrations above 2 per cent agar the general trend of results is a decrease in numbers. The 0.5 per cent agar solidified perfectly, but it allowed the colonies to develop faster, hence at the end of 120 hours they had attained a very large size. This fact would tend to keep down the numbers of bacteria developing on this medium. The lower concentrations of agar appear to be more favorable to certain species than to others, an inspection of Plates I-III will bear out this statement. Such a medium may be uti-

TABLE IV

EFFECT OF AGAR CONCENTRATION ON BACTERIAL COUNTS[1]

Per cent Agar in Media	0.5	0.75	1.0	1.25	1.5	2.0	3.0	4.0
Soil V.....	4.10	4.65	6.40	6.25	7.35	6.50	6.70	6.50
	4.60	4.50	6.00	6.50	7.50	6.75	6.60	6.50
	4.65	5.00	5.90	6.85	7.65	7.25	6.50	7.50
	4.50	4.00	5.40	7.75	6.50	6.40	7.00	8.00
Average	4.46	4.54	5.93	6.81	7.25	6.75	6.70	7.13
Soil VI.....	7.00	7.00	9.50	13.50	15.00	12.50	14.50	14.50
	6.80	7.00	9.20	13.00	15.10	14.20	13.50	13.00
	7.00	7.20	10.00	13.50	13.00	13.70	13.50	14.00
	6.40	lost	8.10	14.40	16.00	13.00	lost	13.30
Average	6.80	7.07	9.20	14.10	14.78	13.35	13.83	13.70

[1] Bacteria calculated in millions per gram of soil.
Dilutions 1:50,000.

lized in the study of the cultural characteristics of certain bacteria which form colonies too minute to be easily studied on more concentrated media. One per cent concentration of agar allowed more and larger colonies to develop, but it is open to the same objections as the 0.5 per cent agar. It does not allow a maximum colony development of the bacteria which are present. The species, too, seem to be restricted, as in the lower concentrations. There is but little difference between the 1.5 per cent and 2.0 per cent agar so far as counts alone are concerned, but since the colonies are larger, the species apparently the same, the greater ease in pouring and counting the plates, and finally the use of less agar, lead the author to the conclusion that 1.5 per cent agar is the best under the conditions of the experiment. Concentrations higher than 20 gm. of agar per liter of medium (2 per cent) form such a hard mass in the plates that the colonies are very small and some fail to develop at all. Such a medium may

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be of value in cases where long storage is necessary, as it does not lose water by evaporation very readily. Other objections to high agar concentrations are the difficulty in filtration, pouring the plates, counting and identifying the species, and finally the added expense of the agar itself.

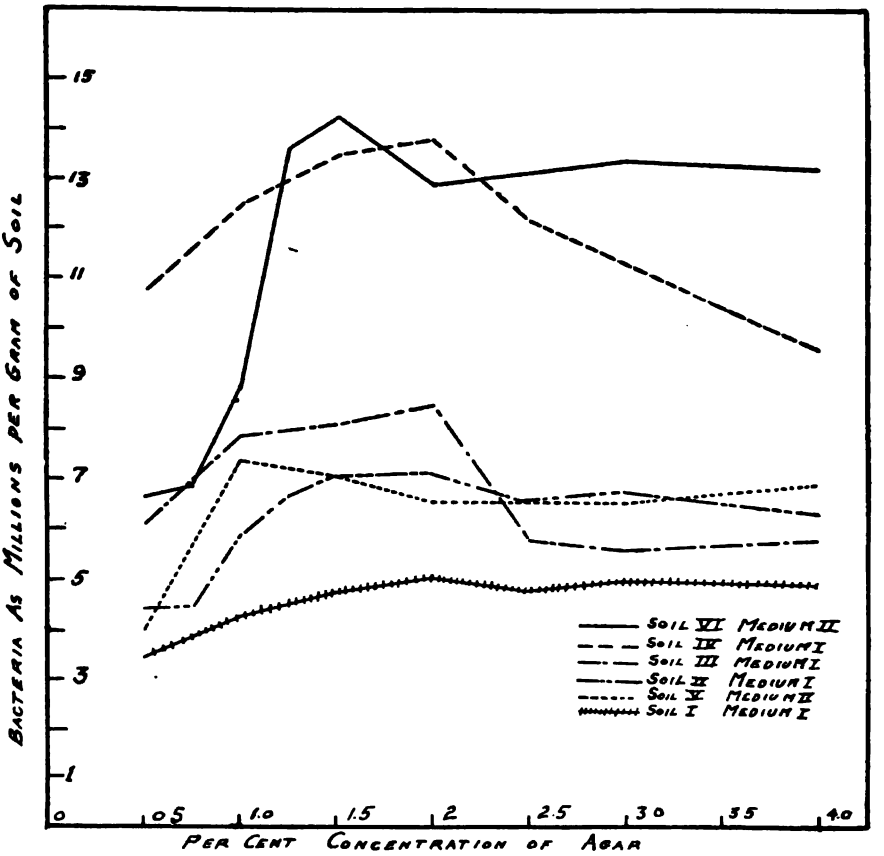

Fig. 1.—Diagram showing the effect of agar concentration on bacterial counts.

The same results hold with both the media tried, and with the several soils. Additional concentrations of 0.75 per cent and 1.25 per cent were tried in the examination of Soils V and VI. The 1.25 per cent concentration gave very good results, the colonies being of good size, the species well represented and the medium firm and hard. There is little difference between 1.25 per cent and 1.5 per cent agar. The 0.75 per cent agar has the same undesirable features as the 0.5 per cent, yet it may be useful in the determination of cultural characters of certain bacteria, and especially the actinomyces, streptothrices and some fungi. Agar of low concentrations may have a practical application in the culture of *Pseudomonas radicicola* for inoculation purposes, as a much larger growth will develop than on more concentrated substratum.

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To test out this point further, pure cultures of *Bacillus coli, Bacterium mycoides* and *B. subtilis* were inoculated on agar slants in test tubes and on streaks in Petri dishes. In the case of all three bacteria the growth was more abundant on the media of low concentration of agar, but this heavy growth took place at the expense of the latter's characteristic appearance. *B. coli* incubated at 30° C. grew best at 1.5 to 2.0 per cent agar concentration, the growth becoming more uncharacteristic as the stiffness of the medium increased. *Bact. mycoides,* on the other hand, was much more characteristic in appearance at lower concentrations of 0.75 and 1.25 per cent agar. *B. subtilis* grew equally well on all the media, but the wrinkly appearance did not show up well except at concentrations of 1.5 per cent or over.

Summary. On the whole a medium having an amount of agar equivalent to 12.5 to 15 gm. per liter has been found to be the best in this series for the following reasons.

1. It permits of a maximum development of colonies. 2. The species are better represented than on lower concentrations and they form more characteristic colonies than at higher concentrations. 3. The medium is more easily filtered, melted and poured into plates than agar of greater concentrations. 4. The well formed colonies permit of very accurate counting. 5. Since agar has been shown to contain many impurities, these are correspondingly reduced with the concentration. 6. Less agar is required.

Low concentrations of agar may be of value in the study of cultural characteristics of certain microörganisms, due to large colony development.

High agar concentrations may be utilized, either where long storage is necessary, or for very slow growing organisms.

The Influence of the Acidity of the Medium on the Number of Bacterial Colonies Developing on Plates

The question of the proper reaction of media for bacteriological examination of soil and water is of great importance. Some standard reaction should be used in the ordinary examination of soil, milk and water for microörganisms. Of course the optimum reaction is not the same for the different kinds of microörganisms, as bacteria, yeasts and fungi, and even different species of bacteria or fungi thrive best in media of a particular acidity or alkalinity. For example *Bacterium mallei* grows best at an acidity of about 2.5 per cent. Most bacteria could not endure so much acid and would not develop on such a medium. On the other hand *Bacterium anthracis* and most of the streptothrices prefer an alkaline medium. The problem is then one which is impossible except in so far as a medium of such reaction must be used which will allow the development of the greatest number of species from the substance under examination.

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An acidity of + 1 (i. e. 10 c.c. Normal HCl per liter of media) is most often used by bacteriologists. This is the reaction recommended by the American Public Health Association Committee on Methods for the Examination of Water and Sewage (41). They allow an error of ± 0.2 in the final reaction. Considerable work has been done on the question of reaction of media. Most of it is either antiquated, as for instance the use of litmus or other unreliable indicators in the titration, or else of minor importance. Only a small part of the literature will be reviewed here.

Some of the investigators recommending alkaline media are Hiltner and Stömer (20), Thomann (46), Deeleman 8), and Hesse and Niedner (18). In their opinion an alkaline medium is preferable in soil or water examination. Others preferring neutral media are Heim (16) and Schultz (40). Lipman (24) and Christensen (4) believe slightly alkaline media are best for Azotobacter, while Wright (49) avers anaerobes as a rule prefer a medium which is near the neutral point. Temple (44) states that the optimum reaction for media in soil work is between the neutral point and 0.5 per cent acid. Cook (7) uses a reaction of+ 0.25 in his urea—NH_4NO_3 agar with excellent results. Conn (6), who has made a very thorough study of media for soil examination, proposed a sodium asparaginate agar, with a reaction of 0.8 per cent HCl. He showed that a medium with an acidity of 0.8 per cent HCl gave about double the number of colonies as one with an acidity of 1.5 per cent HCl. He did not try any acidities below 0.8 per cent for fear of volatilizing some of the ammonia from the NH_2 H_2PO_4 which he used in this medium. For his soil extract gelatin, however, a reaction of 0.5 per cent acid was used.

Lipman and Brown (25) showed that the neutralization of a part of the acidity of their synthetic agar, greatly reduced the number of colonies developing on the medium. This medium owes its acidity mainly to the K_2HPO_4 which is used. The author has determined the reaction several times with the result that an average acidity of about 0.4 to 0.5 per cent was found. A simple change of reaction does not satisfactorily account for the large decrease found, hence some other factors must enter into the problem. Petri and Maassen (32) have pointed out that tertiary phosphates are harmful to most bacteria, and assuming the following equation, tertiary phosphates are formed when the acidity is neutralized;

$$NaOH + K_2HPO_4 = NaK_2PO_4 + H_2O$$

or since $MgSO_4$ is present possibly some Mg would replace the alkalies with the formation of a magnesium phosphate, which may be precipitated and hence would be of no value to the bacteria. Their results at least may be explained on the theory either that the tertiary phosphates are deleterious to bacteria or else that the precipitation of some of the nutrients occurs.

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Series I

In order to determine approximately the optimum reaction of agar media for the purpose of obtaining the largest bacterial counts on plates, a series of soils and waters were quantitatively examined by the plate method. On account of the complexity of salts present in synthetic media, an ordinary peptone—KCl agar (Medium I) was used in this work.

Each batch of media was carefully prepared at the same time, thoroughly mixed, placed in flasks and the reaction adjusted by the titration of 10 c.c. of the medium at a temperature of 50° C. with N/20 NaOH, phenolphthalein being used as the indicator. The temperature at which the titrations were made was 50° C. because peptone solutions are decidedly more acid at higher than at lower temperatures. Some error is introduced by titrating at 50° C. but the change in acidity from 50° C. to incubation temperature is slight compared with that between 100° C. and 50° C.

Media differing by 1 per cent were made up from — 5 per cent NaOH to + 5 per cent HCl. It was found that agar containing more than 2 per cent HCl fails to solidify if it is sterilized in an autoclave under pressure. To overcome this difficulty the following procedure was used. The medium, as soon as it was made up and while still boiling hot, was adjusted in reaction. The average of duplicate 10-c.c portions removed, cooled to 50° C. and titrated, was used to determine the reaction. After adjusting the reaction of all the flasks and thoroughly shaking, the flasks were cooled to 45° C. Then 9-c.c. portions were pipetted out into the sterile Petri dishes containing the inoculum. The usual precautions against contamination were observed during the whole process. By this procedure the agar solidified very well up to 4 or 5 per cent acid. This method however was utilized only in the case of media which would not otherwise harden. Five plates were poured, the four best checks being reported.

Discussion of Results. Upon examining the curves in figure 2 compiled from Table V it is seen that their general appearance is the same, i. e. three out of the four reach a maximum at the neutral point, the fourth (Soil IV) reaching its greatest height at + 1. This shows that the best colony development takes place at a neutral or slightly acid reaction. Very few bacterial colonies developed at acidities greater than + 2, yet the small but fairly constant number that were invariably present, appear to constitute a peculiar group of acid-resistant bacteria. The + 5 medium was rather crumbly and soft, making the colonies difficult to see and for this reason too much emphasis cannot be placed on the number of colonies found on this medium. An acidity of 1 per cent inhibited a maximum colony development in three out of four cases. It is interesting to note that Soil IV, which gave the highest counts at + 1 is very

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acid; this may be only a coincidence, but it is the only soil where the + 1 medium is superior to the neutral. Fungi and actinomyces were particularly abundant on all the acid media, several species of Trichodermae, Cladosporia, Penicillia and Aspergilli persisting even on the + 4 and + 5 media. Alkaline media although inferior to neutral or slightly acid do not appear to be as toxic, as a whole, as the more acid media. The size of the colonies appears to increase as the neutral point is approached from either direction. Few colonies develop on — 4 and — 5 media. The

TABLE V

INFLUENCE OF REACTION OF MEDIA ON BACTERIAL COUNTS

SERIES I

Bacteria per gram of soil or per cubic centimeter of water

Reaction	+5.0	+4.0	+3.0	+2.0	+1.0	Neut'l	—1.0	—2.0	—3.0	—4.0	—5.0
Soil II...	0	100	400	600	2,500	5,000	3,500	2,250	1,700	1,250	500
Dil. 1:50,000	0	200	350	600	3,000	4,250	3,700	2,050	1,450	1,400	700
	50	200	200	400	2,800	3,600	4,050	2,500	1,750	1,000	700
	100	250	350	600	2,350	4,500	3,750	1,700	1,700	1,100	800
Average ...	75	187	325	550	2,660	4,340	3,750	2,130	1,650	1,190	675
Soil I...	50	100	100	400	2,050	2,600	1,350	950	450	100	100
Dil. 1:50,000	0	100	100	300	2,200	2,500	1,550	1,000	500	150	150
	0	50	200	450	2,050	2,550	1,850	1,350	400	300	50
	0	0	100	500	2,500	2,320	1,800	1,050	600	100	150
Average ...	13	63	125	413	2,200	2,492	1,640	1,090	587	163	112
Soil IV...	0	0	150	350	3,500	2,500	2,300	1,300	650	300	100
Dil. 1:50,000	0	0	100	200	3,450	2,800	1,900	1,300	750	250	150
	0	0	100	250	3,000	2,750	2,000	1,000	500	400	50
	0	50	50	150	3,200	3,300	1,600	1,100	900	400	50
Average ...	0	13	100	238	3,300	2,840	1,950	1,180	700	338	88
Water I											
Bac. per c.c.	0	50	60	160	240	600	160	80	80	60	20
Dil. 1:20 ..	0	20	40	180	400	540	200	120	40	60	40
	0	30	60	200	300	460	240	100	40	40	40
	20	40	lost	200	340	440	280	60	80	20	0
Average ...	5	35	55	190	350	410	220	90	60	45	25

Note:—Thousands omitted in case of Soils I, II, and IV.

addition of much alkali to agar causes it to darken, thus making the counting of the plates difficult. Streptothrices grow better on neutral and alkaline media while on the other hand alkalies inhibit the development of fungi. In the three cases where the neutral media gave the highest counts the increase over the 1 per cent acid agar is about 20 per cent. The size of the colonies on the very acid or alkaline media is as a rule very small, the size, pigmentation and the individual colony characteristics become more conspicuous near the neutral point.

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Series II

To check up this work and confine the limits of acidity and alkalinity more closely, another series of plates was poured, in which the reactions used were from + 1.5 to — 0.5.

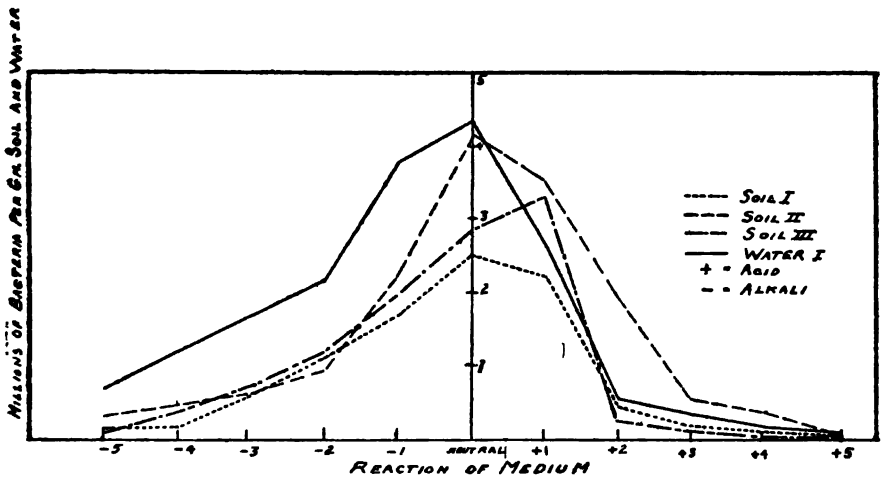

Fig. 2.—Diagram showing the influence of reaction of media on bacterial counts (Series I).

The results follow in Table VI, the averages being graphically represented in figure 3.

Discussion of Results. The data contained in Table VI and the graphs drawn from this in figure 3, confirm in general the first experiment. Beginning with an alkalinity of — 0.5 there is a large increase in the number of colonies per plate, up to the neutral point, then there is a comparatively constant decrease, except in one case, from neutral to + 0.5, but all acidities tested greater than this, are decidedly injurious to the development of bacterial colonies. There is not a very marked difference in the numbers of colonies on neutral and + 0.5 media. Averaging the results of the three soils the neutral medium shows an increase in bacterial colonies over the + 1 per cent acid medium of 18 per cent. In the case of the two waters there is a 12 per cent increase for the neutral medium over the + 0.5 and about 17 per cent over the + 1.0 media.

Water Sample III shows a depression at + 0.5, but this is plainly an exception to the general trend of the results.

From this experiment the conclusion may be drawn that with the media used, most bacteria from soil or water develop best at a neutral or slightly acid reaction. Probably + 1 is too acid for the development of maximum numbers of bacteria. This together with the more characteristic growth of colonies and greater freedom from fungi, are advantages to

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be gained by the use of a neutral or slightly acid medium. It may be that lack of systematic work along this line has led to the usual recommendation of media which is too acid to give the best results. Also, as has been pointed out before, peptone media have a greater apparent acidity in hot than in cold solutions, hence the cooled solution is considerably less acid than is supposed from the titration at the higher temperatures.

TABLE VI

INFLUENCE OF REACTION OF MEDIUM ON BACTERIAL COUNTS[1]

SERIES II

Reaction	+1.5	+1	+0.5	Neutral	—0.5
Soil III.....	1.30	4.10	5.00	5.40	1.50
	1.65	4.10	4.90	5.50	1.80
	1.25	4.90	4.80	6.25	1.45
	lost	4.50	5.50	5.25	2.00
Average	1.40	4.40	5.05	5.58	1.70
Soil V.....	2.95	4.50	5.25	5.50	3.60
	3.00	4.30	5.05	5.60	3.20
	3.25	3.95	5.50	6.00	3.00
	2.60	4.70	4.70	6.35	3.20
Average	2.95	4.36	5.13	5.86	3.25
Soil V.....	1.50	2.10	2.50	2.10	1.30
	1.30	2.00	2.60	2.00	1.25
	1.15	1.60	2.30	1.80	1.50
	1.25	1.90	2.80	2.50	1.50
Average	1.30	1.90	2.55	2.10	1.38
Water II.....	600	850	980	1060	750
Dilution 1:10	500	700	850	1020	800
	480	720	900	950	760
	520	lost	800	920	680
Average	530	760	880	990	750
Water III.....	340	760	850	1040	450
Dilution 1:10	360	920	780	980	400
	330	900	760	900	470
	330	870	780	940	470
Average	340	860	790	970	450

[1] Millions omitted in case of Soils I, III and V.
Soil dilutions 1:50,000.

The medium which was titrated boiling hot and adjusted to a reaction of + 1, on cooling to 23° C. was found to be 0.75 per cent acid. In special cases as in water examination, where bacteria of the "coli" group are especially sought for, an acidity of + 1 may be justified. Since practi-

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cally all waters are alkaline and most soils only slightly acid, the above findings seem to be in harmony with the natural conditions (See Plate IV, fig. 1).

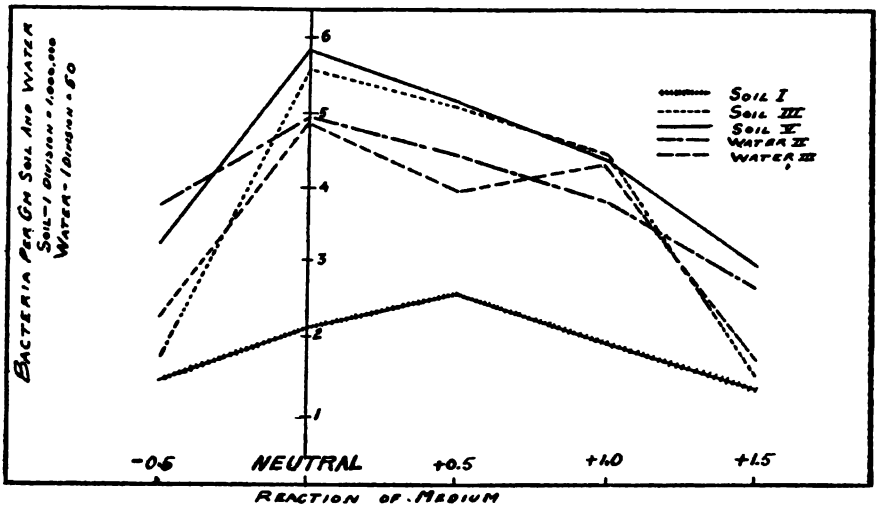

Fig. 3.—Diagram showing the influence of reaction of media on bacterial counts

Series III

A number of bacteria and fungi were inoculated on agar slants and as plate streak cultures, made up with media of varying reaction. Table VII gives the relative growth of the several bacteria tested. The data on the fungi are given later.

TABLE VII

INFLUENCE OF REACTION ON GROWTH OF BACTERIA

Reaction	Bact. mycoides	B. subtilis	B. fluorescens liquefaciens	B. coli	B. prodigiosus
—4	No growth	Moderate	No growth	Very feeble	Feeble
—3	Very feeble	Moderate	No growth	Very feeble	Moderate
—2	Feeble	Vigorous	Feeble	Feeble	Moderate
—1	Moderate	Vigorous	Moderate	Moderate	Vigorous
N	Vigorous	Vigorous	Vigorous	Moderate	Vigorous
+1	Moderate	Vigorous	Moderate	Vigorous	Moderate
+2	Feeble	Vigorous	Feeble	Moderate	Feeble
+3	Very feeble	Feeble	No growth	Feeble	Very feeble
+4	No growth	Feeble	No growth	Very feeble	No growth

Although the table gives only a limited amount of data, yet it is interesting in that it shows the most favorable reaction for the growth of the organisms tested. In most of them there is a considerable range where the growth is good, for example *B. subtilis* grew on all the media tested. *Bact. mycoides* and *B. prodigiosus* seemed to prefer neutral or

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slightly alkaline media, *B. fluorescens liquefaciens* grew best on neutral, and *B. coli* best on media of an acid reaction. Another point brought out by this experiment is the change in character of the growths, pigment production, etc., which occur when the bacteria are grown on media of different reactions. For instance *B. prodigiosus* produced a very dark red pigment on alkaline and neutral media, and at acidities over 2 per cent there was little or no color.

The well known thick wrinkly growth of *B. subtilis* appeared only at reactions between -3 and $+2$. On the more alkaline media it was smooth and slimy, while on very acid media the growth was scanty and had a dried appearance. *B. coli* is adapted to a wide range of acidity and alkalinity, the growth probably being most vigorous at $+1$. *Bact. mycoides* grew poorly at acidities greater than $+1$, the growth being of a dirty gray color, amorphous in structure and entirely devoid of the characteristic rhizoidal appearance. Microscopic examination of the cultures revealed no striking morphological changes, except that in the case of *B. subtilis* after 10 days there were vegetative forms of the organism only on the -1 and neutral media.

These results show the importance of making culture media always of the same reaction, due to the variability of many bacteria to slight reaction changes. Lipman (24) has suggested a practical application of this phenomenon, in the approximate determination of soil acidity.

IV

Influence of Reaction of the Medium on the Number of Fungi Developing on Plates

It is generally conceded that fungi desire a more acid medium for best development, than do bacteria. But little experimentation has been conducted on the effect of reaction on fungi. Thom (45) has shown that most species of Pencillium do not thrive well on alkaline media, but that the best reaction is from neutral to an acidity of N/10 HCl. Different acids have very different actions on fungi. Traaen (47) states that an acidity of from N/150 to N/50 is most suitable for the majority of fungi. Trichoderma is especially resistant. H_2SO_4 is not as toxic as HCl which in turn is not as toxic as HNO_3. Marchal (28) states that soils having a reaction from neutral to alkaline are not favorable for fungi. Kopeloff (23) found that certain species of Rhizopus, Zygorhynchus, and Penicillium have as a rule a narrow range of reaction tolerance as regards ammonification activities. This lies between the neutral point and an acidity equivalent to 2000 pounds CaO per acre. Greater acidities than this caused a depression in ammonifying power, as did an increase in alkalinity. Coleman (5) working with a number of soil fungi found that they were adapted to a wide range of reaction tolerance, but that their activities were suppressed in alkaline or very acid media.

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The medium used in the present work to determine the best reaction for growth of soil fungi is one proposed by Mr. G. P. Koch, one of the author's colleagues, in unpublished data. It consists of,

Agar—20 gm.	K_2HPO_4—.25 gm.
Glucose—20 gm.	$MgSO_4$—.25 gm.
Peptone—5 gm.	..Distilled H_2O—1000 c.c.
NH_4NO_3—1 gm.	Reaction + 1

The reaction was varied from — 1 to + 2 by steps of 0.5 per cent. The + 1.5 and + 2 media did not harden after sterilization, hence the method already described to cause the agar to harden was resorted to. This medium gave good results, the colonies of fungi being well separated, and distinct. The growth of mucors and streptothrices was also inhibited. The counts given in Table VIII, and represented in figure 4 were made after five days' incubation.

TABLE VIII

INFLUENCE OF REACTION OF MEDIUM ON COUNTS OF SOIL FUNGI[1]

	+2.0	+1.5	+1.0	+0.5	Neutral	—1.0
Soil IV.....	120	150	160	70	90	50
Dil. 1:10,000	140	120	120	80	60	40
	110	160	150	90	50	30
	120	140	120	80	60	lost
Average	123	143	138	80	65	40
Soil II.....	40	80	100	90	40	40
Dil. 1:10,000	60	60	90	60	60	40
	90	90	70	60	40	60
	Mucors	Mucors	80	60	60	Mucors
Average	63	77	85	68	50	47
Soil V.....	70	130	120	100	90	70
Dil. 1:10,000	90	130	120	90	60	50
	110	140	150	100	90	30
	80	100	110	lost	90	50
Average	88	125	125	97	83	50

[1] Fungi represented as thousands per gram of soil.

Discussion of Results. Although somewhat incomplete, these results show that the optimum reaction for the development of soil fungi is more acid than the bacterial optimum. There is little difference between the counts on the media whose reactions were respectively + 1 and + 1.5. Alkaline or neutral media do not seem to be as favorable as those with an acid reaction. An acidity of + 2 is too high and + 0.5 is too low for a maximum development of colonies as a glance at the curves will indicate.

It is generally believed more fungi are present in acid soils than in those of a neutral or alkaline reaction. Soil IV is extremely acid while

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Soils II and V are nearly neutral. The data seem to support this belief. As in the experiment with bacteria, a number of genera of soil fungi were tested with media of varying reactions. Table IX gives a summary of the results.

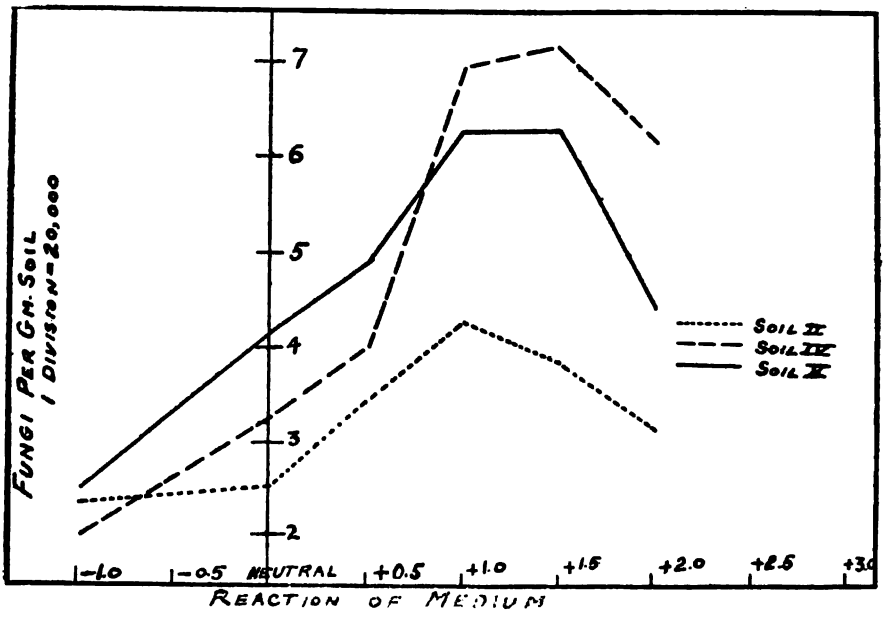

Fig. 4.—Diagram showing the influence of reaction of media on counts of soil fungi.

As a whole the acid media were the more favorable for all the fungi tested. *Aspergillus niger* grew and sporulated at all the reactions at

TABLE IX

INFLUENCE OF REACTION ON GROWTH OF SOIL FUNGI

Reaction	Aspergillus niger	Cladosporium epiphyllum	Penicillium viridicatum W.	Trichoderma (sp. III)
—5	Very feeble	Very feeble (pigment)	No growth	.....
—4	Feeble	Feeble (pigment)	No growth	Feeble
—3	Feeble	Moderate (deep pigment)	No growth	Moderate
—2	Moderate	Vigorous (pigment)	Moderate	Vigorous
—1	Moderate	Vigorous (pigment)	Vigorous	Vigorous
N	Vigorous	Vigorous (pigment)	Vigorous	Vigorous
+1	Vigorous	Vigorous (pigment)	Vigorous	Vigorous
+2	Vigorous	Moderate (pigment)	Moderate	Vigorous
+3	Moderate	Moderate (slight pigment)	Moderate	Feeble
+4	Moderate	Moderate (very slight pigment)	Feeble	Very feeble
+5	Moderate	.....	.....	.....

which it was grown. *Penicillium viridicatum* W. was also very resistant to acid but sensitive to alkali. *Cladosporium epiphyllum* produced a dark coloration of the media, which became fainter as the acidity increased.

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Many instances are on record where fungi have been observed growing in fairly concentrated acid solutions. An instance of a Penicillium apparently thriving in a bottle of N/10 HCl was noticed by the author. Such phenomena may be explained on the theory that the fungus uses the acid only as a medium in which to develop, and obtains its food from the foreign substances which are present.

Summary of Acidity Experiments

Under the conditions of the experiment:

1. The optimum reaction for the development of maximum numbers of soil and water bacteria is from the neutral point to an acidity of + 0.5.
2. For soil fungi the optimum acidity is from + 1 to + 1.5.
3. Many soil bacteria and fungi are able to develop at comparatively high concentrations of acid and alkali.
4. On the whole, acidities of over 2 per cent seem to be more inhibitive to microbial growth than alkaline media of a similar reaction.
5. Streptothrices are favored by an alkaline medium.
6. The size of the colonies of soil bacteria appears to increase as the neutral point is approached from either direction.
7. The growth characteristics and chromogenesis of certain bacteria are much changed when growing on unfavorable media.
8. Some fungi have a remarkable ability of thriving in very acid or alkaline media, the character of the growth of the organism, being as a rule, less subject to variability than is the case of bacteria.

V

Effect on Bacterial Counts of Sterilization of Agar Media at Different Pressures and Temperatures

The question often arises whether the different sterilization pressures obtained in an autoclave, have any influence on the bacterial count obtained from the media so treated. It is the common idea that there is little if any harm done to ordinary agar media by sterilizing in an autoclave, provided the temperature reached is not too high or the period of sterilization process not too long continued. Probably the most common recommendation is to heat the media in an autoclave at 15 pounds pressure for 15 minutes. Of course gelatin, milk or blood serum media must be treated differently, but only agar media are here discussed. Moore (31) states that media sterilized under pressure in an autoclave is detrimental to the growth of certain pathogenic bacteria. Cook (7) found no difference in the counts obtained with media which had been sterilized at flowing steam and by autoclaveing for 15 minutes at 15 pounds pressure. Most bacteriologists agree that long continued heating is injurious either because of chemical changes wrought in the medium or to precipitation of some of the nutrients.

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In the following experiment four pressures were used at which to sterilize the media. These are:

(1) Boiling water, i. e. atmospheric pressure.
(2) One atmosphere.
(3) Two atmospheres.
(4) Three atmospheres.

The sterilization in boiling water was accomplished by simply immersing the tubes of media in a vessel containing the hot water for one-half

TABLE X

INFLUENCE OF STERILIZATION OF MEDIA AT VARYING PRESSURES ON BACTERIAL COUNTS[1]

	Medium I				Medium II			
	Boiling Water	1 Atmosphere	2 Atmospheres	3 Atmospheres	Boiling Water	1 Atmosphere	2 Atmospheres	3 Atmospheres
Soil I.....	4.30	3.70	3.65	3.10	6.50	6.10	4.75	2.10
Dil. 1:50,000	4.50	3.90	3.50	3.35	6.85	6.10	4.60	2.00
	4.75	4.00	3.35	4.05	6.45	6.20	5.00	2.30
	5.00	3.50	3.85	3.65	5.85	6.25	4.55	1.95
Average	4.51	3.78	3.59	3.54	6.41	6.16	4.75	2.09
Soil II.....	3.25	2.80	2.90	3.30	6.20	5.85	5.45	3.50
Dil. 1:50,000	3.60	2.50	3.00	3.50	6.25	6.00	5.55	3.25
	3.60	2.70	2.50	3.75	6.05	5.65	4.80	3.50
	3.25	2.85	2.60	3.70	6.60	5.35	4.70	3.20
Average	3.43	2.71	2.75	3.56	6.28	5.71	5.13	3.36
Soil III.....	6.35	5.65	4.55	6.15	7.60	6.40	5.40	2.75
Dil. 1:50,000	6.50	5.25	4.75	6.30	7.45	6.30	5.15	2.40
	6.00	5.20	5.65	5.90	7.10	6.05	5.00	2.20
	6.50	5.40	5.00	5.55	6.50	5.75	4.75	2.20
Average	6.34	5.38	5.24	5.98	7.16	6.13	5.08	2.39
Water III...	550	500	390	395	420	400	325	255
Dil. 1:5	530	540	400	390	400	375	300	250
	495	550	355	425	375	350	315	270
	520	500	350	390	410	400	345	230
Average	524	523	374	400	401	381	321	251

[1] Millions of bacteria per gram of soil for Soils I, II and III. Number of bacteria per c.c. of water for Water III.

hour on two successive days. A 15-minute period was used in sterilizing in the autoclave. Three soils and a sample of water were used as a source of inoculating material. Two media were used, namely, a peptone —KCl agar (I) and, Lipman and Brown's modified synthetic agar (II). All media were treated in precisely the same manner except in the method of sterilization. Table X gives the results obtained in this experiment.

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The results are represented graphically in figure 5. Photographs of the plates are given in Plate IV (fig. 2) and Plate V (fig. 1, 2).

Discussion of Data and Curves. Since the results obtained with the two media are quite different each will be discussed in turn. Medium I (peptone) gives in all cases the highest counts, when the sterilization is accomplished by boiling water. This is more marked in the three soils tested than in the water sample, which shows practically no difference in counts between sterilization with hot water or at a pressure of one atmosphere. The average decrease in the number of colonies due to sterilization at one atmosphere is approximately 14 per cent. There is a further decrease at 2 atmospheres, but at 3 atmospheres, strange to say, in three of the four samples tested there is an increase in the number of colonies developing. Such results are difficult to explain satisfactorily unless we consider

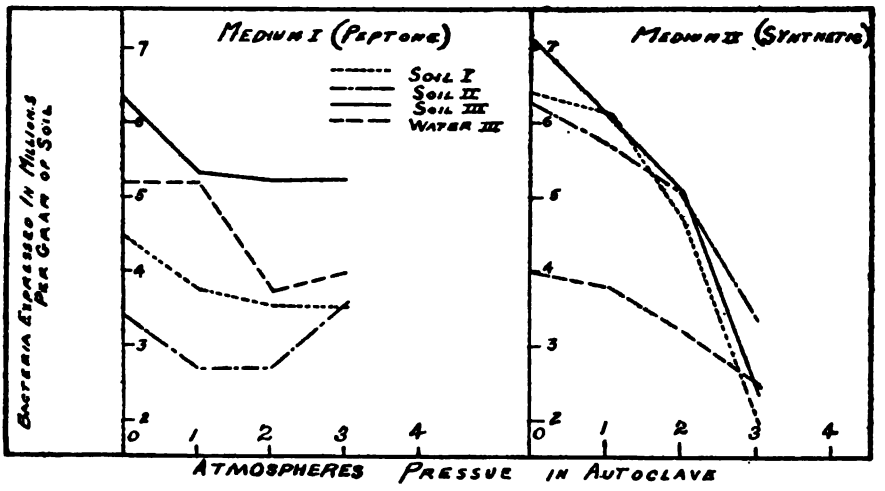

Fig. 5.—Diagram showing the influence of sterilization at different temperatures on bacterial counts.

that peptone is broken down into simpler amino-acids or changed in some way by the high heat obtained at 2 atmospheres pressure in the autoclave, and then on raising this to 3 atmospheres, another favorable change takes place making some of these compounds more available, or else less toxic. From the data at hand it appears that sterilization at 100° C. at atmospheric pressure is preferable for peptone media, but it is possible that if pressures below one atmosphere were used, or if the time were lessened, the pabulum may not be rendered less satisfactory for bacterial development.

Passing on to Medium II (synthetic), it is seen that there is a gradual and almost constant decrease in the bacterial counts as we pass from a sterilization temperature of 100° C. to one much higher which is obtained at a pressure of 3 atmospheres in an autoclave. This seems logical, as it is likely that the composition of the medium becomes considerably

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altered at the high temperatures, rendering the nutrients less available or more toxic for bacteria.

Summary. Although these results are by no means conclusive, still the evidence at hand is decidedly in favor of a sterilizing temperature of 100° C. In the experiment this was kept up for 30 minutes on 2 successive days. The sterilization was complete.

The high temperatures produced at pressures of over one atmosphere in an autoclave render the media unfavorable for bacterial growth. This experiment also shows the great superiority of the synthetic over the peptone medium.

VI

Effect of Storage of Media on Bacterial Counts

Much has been said but little done on the question of the deterioration of culture media with age. It is generally supposed that media do deteriorate when stored for a period of time but little data of real value are available. Conn (6) showed that soil extract gelatin may change greatly over night, and considers that under certain conditions agar media may also change in a longer or shorter period of time. Petri and Maassen (32) state that media should be well plugged and stored in the dark, as light and oxygen have an injurious effect on it. Ravenel (36) claims media may be preserved several months if tin-foil is placed over the top of the tubes or flasks. Bioletti (1) recommends using an antiseptic-treated cotton plug covered with a rubber cap. Pierce (33) reports that he finds the method of sealing tubes of media with paraffin of much value in preserving cultures. Hill (19) states that a medium should not be stored in large quantities as it is necessary to sterilize it every time a portion is withdrawn, thus injuring it.

Dipping the plugs of the tubes or flasks of media in paraffin has often been used to prevent evaporation. Also humidors where a saturated atmosphere is preserved have been proposed for storing media.

Many isolated filtration plants and laboratories buy their media in large lots which may be stored for several months. Often in the laboratory it is desirable to know if it is allowable to compare counts from one batch of medium with another batch prepared some time before. Also the question arises as to whether it is proper to use the same batch of medium in a long continued experiment.

To throw light upon some of these points an experiment with two media namely: (I) Peptone—KCl agar and (II) Lipman and Brown's modified synthetic agar, was begun. Medium I was stored in the test tubes and flasks in a cool, dark place, and tested monthly against freshly made media. Tubes both with paraffined plugs and with unparaffined plugs were used. The plugs which were treated with paraffin were cut off close to the mouth of the tube, and dipped into the molten wax. Well

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made, close fitting plugs were used to plug the other half of the tubes. The flasks used were of 500 c.c. capacity, each containing about 350 c.c. of medium. In order to make sure that each of these media was uniform throughout, it was all prepared in one large batch, and thoroughly mixed before tubing. Especial care was taken to test the reaction and to adjust it in the case of Medium I, both before and after sterilization.

Tests were made at intervals of about a month for six months. As it was contemplated that if any differences in counts were obtained during the course of this experiment, such would probably be small, ten duplicate plates were poured of *each* medium. The experimental error method of reporting results is used as where so many plates are poured, but little variation is probable, and a good average is possible. The same ingredients precisely were used in the freshly made media as were present in the original batch; even the tap water was stored in a large bottle. Counts were made after 120 hours' incubation at a temperature of 22° to 23° C. The soil and water samples used are from different sources, but of course the same samples were used in the individual tests.

TABLE XI

WEIGHT OF MEDIUM IN TUBES WITH PARAFFINED PLUGS

Wgt. of Medium	Freshly Prepared Oct. 20 gm.	Nov. 16 gm.	Dec. 15 gm.	Jan. 15 gm.	Feb. 18 gm.	Mar. 18 gm.	Apr. 10 gm.
In 10 Tubes....	93.00	91.00	85.40	81.00	78.80	73.00	70.00
In Tube I....	9.89	9.53	8.51	7.75	6.97	6.01	5.83
In Tube II....	9.91	9.85	9.25	8.80	8.31	7.85	7.61
In Tube III[1]...	.....	.....	.....	.....	.....	.....	.....

[1] Tube was broken.

A series of ten paraffined and ten unparaffined tubes were weighed monthly to obtain the loss of moisture by evaporation. Three tubes from each set were also weighed singly for purposes of comparison. Tables XI, XII and XIII show the results obtained.

The acidity of the various media was also tested from time to time. The data thus obtained may be found in Table XIV.

Tables XI, XII and XIII show that there is a great loss of moisture by evaporation on storing media. The tubes with the unparaffined plugs lost nearly twice as much moisture as the tubes with paraffined plugs. In some cases the former suffered a loss in water content of 50 per cent in 6 months. Although the method of saturating the plugs with paraffin aids in hindering evaporation yet the loss is considerable after a few months. In one month the maximum percentage loss of moisture from any of the tubes was 6 per cent. This is not enough to injure the medium seriously as is shown in Table XIV, the rise in acidity being very small. The acidity of the medium of course increases with the evaporation of water. The

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nutrients too become more concentrated and possibly changed in nature due to long storage. After 6 months the acidity of the medium in the unparaffined tubes had nearly doubled itself. How this effects the bacterial counts is shown in Table XV. The curves in figure 6 show that the increase in acidity is directly proportional to the loss of moisture from the media.

TABLE XII

WEIGHT OF MEDIUM IN TUBES WITH UNPARAFFINED PLUGS

Wgt. of Medium	Freshly Prepared Oct. 20 gm.	Nov. 16 gm.	Dec. 15 gm.	Jan. 15 gm.	Feb. 18 gm.	Mar. 18 gm.	Apr. 10 gm.
In 10 Tubes....	87.00	82.00	72.00	65.00	61.40	50.50	45.00
In Tube I....	10.00	9.65	8.69	8.10	7.23	6.67	6.18
In Tube II....	10.39	9.32	8.24	7.47	6.64	5.95	5.50
In Tube III....	9.49	9.15	8.12	7.36	6.55	5.84	5.40

TABLE XIII

PERCENTAGE LOSS OF MOISTURE ON STORAGE OF PARAFFINED AND UNPARAFFINED TUBES

Length of Storage	27 Days	56 Days	87 Days	121 Days	150 Days	173 Days
	%	%	%	%	%	%
Loss in 10 paraffined tubes.....	2.2	8.0	12.8	15.2	21.5	24.7
Av. loss of 2 paraffined tubes.....	2.0	10.4	16.5	22.8	30.0	32.0
Loss in 10 unparaffined tubes.....	5.6	17.2	25.3	29.5	42.0	48.2
Av. loss in 2 unparaffined tubes...	6.0	16.0	23.2	33.5	39.3	43.0

TABLE XIV

EFFECT OF STORAGE OF MEDIA ON ITS ACIDITY

Length of Storage	Original Acidity	27 Days	56 Days	121 Days	173 Days
	%	%	%	%	%
Av. acidity of 3 paraffined tubes...	1	1.10	1.24	1.42	1.62
Av. acidity of 3 unparaffined tubes.	1	1.13	1.38	1.61	1.86
Medium I stored in flasks.....	1	....	....	1.20	....
Medium II stored in flasks.....	0.4	....	....	0.60	....

Each figure in Table XV represents ten plates except in Water Sample III and in column 7, where the averages are the results of five plates only. The experimental error was calculated from the formula

$$E = \pm .6745\sqrt{\frac{\Sigma D^2}{n\,(n-1)}}$$

E = Probable error.

D = Deviation from the average.

n = Number of determinations.

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Discussion of Tables XV and XVI and Curves. The foregoing data show that the media which were used in this experiment deteriorate if stored for any great length of time. If the plugs of the tubes are not paraffined the medium becomes poor very rapidly. With a peptone agar, in one month a percentage decrease in bacterial counts over the check of 26.8 per cent was noted. Such a difference between two media is enough to make worthless any quantitative work in which they are used. This de-

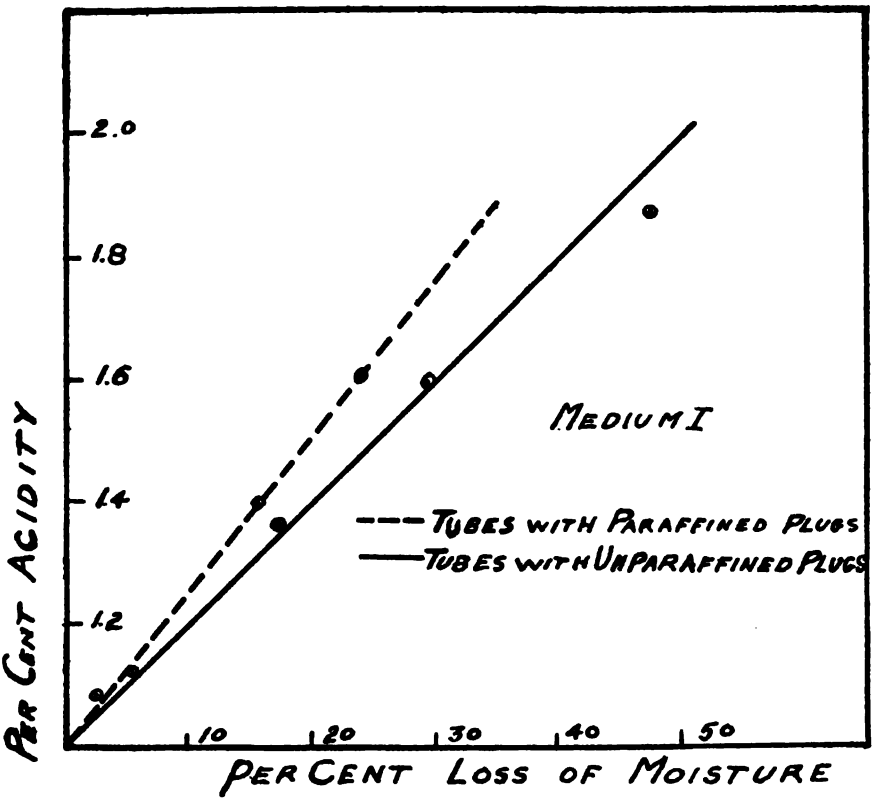

Fig. 6.—Diagram showing the correlation of moisture loss and acidity increase.

terioration becomes greater the longer the time of storage. The curves in figure 7 show that there is apparently a direct ratio between time of storage and bacterial counts.

Dipping the plug end of the tubes in molten paraffin helped to preserve the media to some extent. After two month's storage the percentage decrease in bacterial count was only 12.7, but for longer periods it did not seem to hinder evaporation entirely. The evaporation is approximately one-half that of the tubes with plain cotton plugs.

Flasked media keep better than tubed media, but even here there was a noticeable deterioration after four months. Had the flasks been sealed with paraffin probably the medium would have been preserved much

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longer. Up to three months there is little change in the bacterial counts on peptone agar stored in flasks.

TABLE XV

EFFECT OF STORAGE OF MEDIA ON BACTERIAL COUNTS[1]

Days storage	Material tested	Check Medium I freshly prepared	Medium I Plugs paraffined	Medium I Plugs not paraffined	Medium I Flasks	Medium I. Not paraffined + H_2O[1]
27	Soil V	11.25±.37	8.75±.80	8.31±.13	.....	.....
	Soil VI	15.75±.32	15.20±.95	11.30±.41	.....	.....
	Water III	310±40	310±20	230±30	.....	.....
56	Soil I	1.70±.05	1.40±.03	1.05±.04	1.70±.10	.....
	Soil II	1.85±.09	1.60±.04	1.10±.06	2.05±.13	.....
	Water III	1100±40	1020±60	800±15	1000±30	.....
87	Soil I	1.37±.15	.90±.08	.55±.014	1.24±.10	1.10±.11
	Soil II	3.05±.12	1.52±.02	.97±.02	2.27±.07	2.65±.18
	Water III	590±40	500±27	190±15	740±25	.....
121	Soil I	3.15±.065	1.95±.03	1.335±.12	1.91±.19	1.70±.12
	Soil II	3.25±.075	2.20±.08	1.75±.15	2.05±.15	.....
	Water III	320±16	260±15	200± 8	270±20	.....
150	Soil II	3.30±.13	2.00±.09	1.30±.10	2.90±.20	2.60±.13
	Soil III	7.30±.25	4.80±.21	2.60±.12	5.70±.19	6.1±.22
173	Soil I	2.61±.20	1.30±.09	0.79±.06	.....	Precipit'ed
	Soil II	3.21±.16	1.80±.11	0.92±.09	.....	.....

[1] About 4 c.c. sterile H_2O was added to each tube before melting the medium. Bacteria are expressed in millions per gram of soil.

Since deterioration, evaporation and acidity increases are proportional to the length of storage, and since it has been shown earlier in this paper

TABLE XVI

EFFECT OF STORAGE OF MEDIA ON BACTERIAL COUNTS
Average per cent decrease in bacterial counts over check

Days Storage	Medium I Paraffined plugs	Medium I Plugs not paraffined	Medium I 500-c.c. flasks	Medium I. Plugs not paraffined + 4c.c. H_2O[1]
27	7.9	26.8	0	..
56	12.7	35.2	0.5	..
87	33.2	66.0	5.0	14
121	30.0	53.3	1.37	46
150	36.5	62.0	17.0	18
170	47.0	71.0	....	..

[1] Four c.c. sterile H_2O were added to each tube before melting the medium.

that reaction exerts a tremendous influence on colony development, it seems reasonable to conclude that acidity is here the primary limiting fac-

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tor, with perhaps the high concentration of the nutrients as a secondary inhibiting factor for bacterial growth.

The custom of sometimes adding sterile water to tubes of dried-out agar media furnished grounds for testing out this point in a partial way.

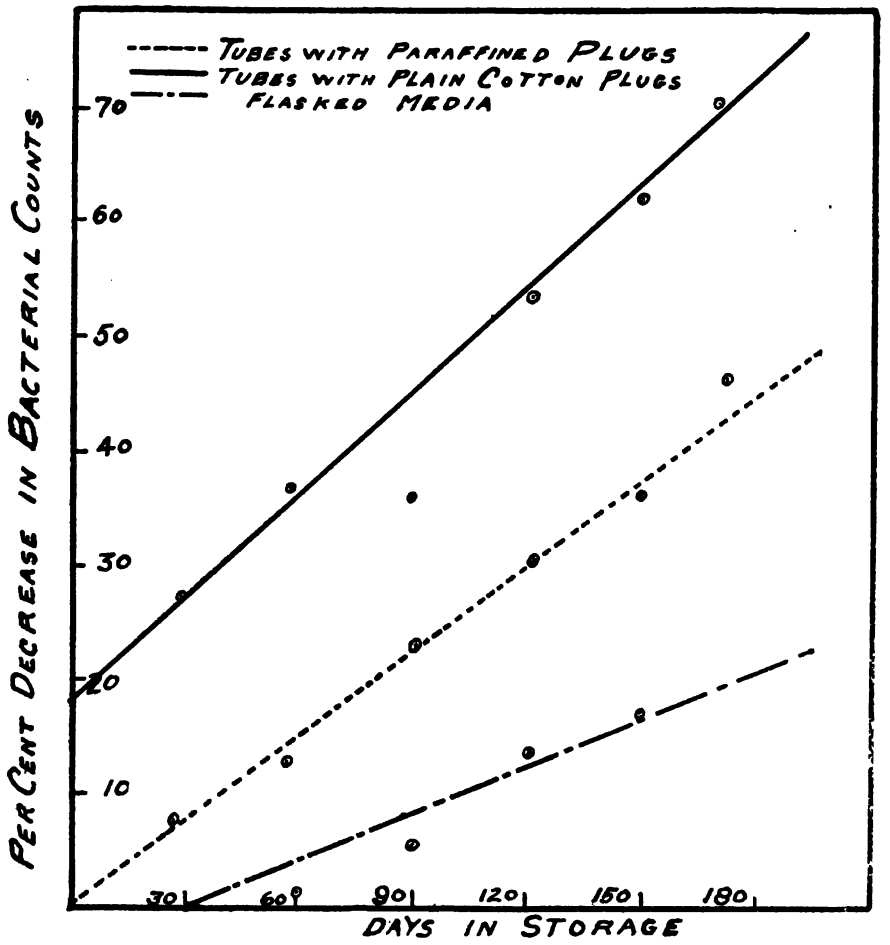

Fig. 7.—Diagram showing the deterioration of media on storage.

The results obtained show that an addition of water aids in restoring the good qualities of the medium, but the restoration is not complete; besides, very often a flocculent precipitate is obtained. The method cannot be recommended except in rough work.

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General Summary

After briefly reviewing the literature touching on the subject it has been pointed out that:

1. Although there are present in agar considerable amounts of soluble nutrients including nitrogenous materials, these are available for microorganisms only to a very limited extent. If the conditions are made favorable however, microbial activity becomes well marked, one of the results being a transformation of a part of the nitrogenous matter into ammonia.

2. The "purified" agar obtained by washing the crude product with dilute acid, and subsequent precipitation of a water solution of the agar by means of alcohol or acetone, is not injured as regards its use as a substratum, since it is firm and does not reduce the bacterial counts obtained from soil, when compared with ordinary agar.

3. A concentration of agar of between 1.25 to 1.5 per cent has been found to be most favorable with the media used, and with soil as the inoculum. Low concentrations of agar are of value in the study of certain microörganisms due to the large colony development. High concentrations are useful where either slow growing organisms are to be cultivated or where the medium is to be stored a long time.

4. Under the conditions of the experiment the optimum reaction of the media tested for the development of maximum numbers of soil or water bacteria, is one between the neutral point and an acidity of $+0.5$ per cent HCl.

5. For soil fungi the optimum acidity is from $+1$ to $+1.5$ per cent HCl.

6. Many soil bacteria and fungi are able to develop colonies at comparatively high concentrations of hydrogen or hydroxyl ions.

7. Streptothrices are favored by an alkaline medium.

8. The size of the colonies of soil bacteria appears to increase as the neutral point is approached from either direction.

9. The growth characteristics and chromogenesis of certain bacteria are much changed when growing on an unfavorable medium.

10. Some fungi have a remarkable ability of being able to thrive in very acid or alkaline media, the character of the growth of the organism being as a rule less subject to variability than is the case with bacteria.

11. With both peptone and "synthetic" agar, the maximum counts were obtained when the media were sterilized in boiling water for one-half hour on two successive days. Sterilization in the autoclave at a pressure of one atmosphere for 15 minutes did not injure the medium to a very great extent, but at higher temperatures and pressures, a serious deleterious effect was noted.

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12. Lipman and Brown's modified synthetic agar (Medium II) gives much higher counts than peptone—KCl agar (Medium I).

13. Peptone media in tubes (Medium I) deteriorates rapidly on storage. This is due to an increase in acidity caused by the evaporation of water from the tubes and consequent concentration of the agar and nutrients. The loss of water by evaporation, the increase in acidity and the decrease in bacterial colonies developing in the medium, are apparently proportional to the length of the storage period.

14. If the plugs are paraffined, the deterioration is not so rapid. Also if the medium is stored in flasks, it may be kept several months without appreciable change.

15. Addition of water to tubes of dried-out media does not restore entirely the original properties of the media, but it aids in correcting the acidity, and hence makes the substratum more favorable for bacterial growth.

16. Synthetic agar (Medium II) also appears to be injured on keeping in storage, but the extent of this injury has not been determined.

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Literature Cited

(1) Bioletti, F. T.
1898. A method of preserving culture media. *In* Jour. Appl. Micros. and Lab. Methods, v. 1, no. 4, p. 72-73.

(2) Biondi, D.
1887. Neue Methode der mikroskopischen Untersuchung des Blutes. *In* Arch. Mikros. Anat., v. 31, p. 103-112.

(3) Brown, P. E.
1913. Methods for bacteriological examination of soils. *In* Ia. Agr. Exp. Sta. Research Bul. 11, p. 379-408.

(4) Christensen, H. R.
1914. Studien über den Einfluss der Bodenbeschaffenheit auf das Bakterienleben und den Stoffwechsel in Erdboden. *In* Tidsskr. Landbr. Planteavl., Bd. 21, p. 321. Also abstract *in* Zentbl. Agr. Chem., Bd. 44, p. 290-296.

(5) Coleman, D. A.
1916. Environmental factors influencing the activity of soil fungi. *In* Soil Sci., v. 2, no. 1, p. 1-65.

(6) Conn, H. J.
1914. Culture media for soil bacteria. *In* N. Y. Agr. Exp. Sta. Tech. Bul. 38, 34 p.

(7) Cook, R. C.
1916. Quantitative media for the estimation of bacteria in soils. *In* Soil Sci., v. 1, p. 153.

(8) Deeleman, M.
1897. Der Einfluss der Reaction des Nährbodens auf das Bakterienwachsthum. *In* Arb. K. Gsndhtsamt., Bd. 13, p. 374.

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(9) DOMINIKIEWICZ, M.
1908. Zur Frage über die Einheit der Zusammensetzung und Herstellungsweisen von Nährsubstraten für Bakterien. *In* Centbl. Bakt. (etc.), Abt. 1, Bd. 47, p. 666-670.
(10) DUGGAR, B. M.
1909. Fungous diseases of plants, p. 33. New York.
(11) EDINGTON, A.
1886. A new culture medium for microörganisms capable of withstanding high pressure. *In* Lancet (London), v. 2, p. 704.
(12) FELLERS, C. R.
1916. The analysis, purification and some chemical properties of agar agar. *In* Jour. Indus. Engin. Chem. (Soon to appear).
(13) FISCHER, H.
1910. Zur Methodik der Bakterienzahlung. *In* Centbl. Bakt. (etc.), Abt. 2, Bd. 25, p. 458-460.
(14) GAZETTI, C.
1911. Importance of the character of the substratum in the biological modifications produced by substances added to culture media, on chromogenic organisms. *In* Arch. Farmacol. Sper. e Sci. Aff., v. 12, p. 25-48.
(15) GRAN, H. H.
1902. Die Hydrolyse des Agars durch ein Enzym. *In* Centbl. Bakt. (etc.), Abt. 2, Bd. 9, p. 562-563.
(16) HEIM, J.
1895. Zur Bereitungsweise von Nährmitteln. *In* Centbl. Bakt. (etc.), Abt. 1, Bd. 17, p. 190.
(17) HESSE, F.
1897. Ueber die Verwendung von Nähragar zu Wasseruntersuchungen. *In* Centbl. Bakt. (etc.), Abt. 1, Bd. 21, p. 932.
(18) HESSE, W., and NIEDNER, —.
1898. Die Methodik der bakteriologischen Wasseruntersuchungen. In Ztschr. Hyg. u. Infectionskrank., Bd. 29, p. 454-462.
(19) HILL, H. W.
1899. A method of making the three principal artificial media. *In* Jour. Appl. Micros. and Lab. Methods, v. 2, no. 3, p. 301-304.
(20) HILTNER, L., and STÖRMER, K.
1903. Studien über die Bakterienflora des Ackerbodens, (etc). *In* Arb. K. Gsndhtsamt., Biol. Abt., Heft 3, p. 447.
(21) HINTERBURGER, A., and REITMAN, C.
1904. Verschiedenes Wachstum des Bacillus pyocyaneus auf Nähragar je nach dessen Wassergehalt. *In* Centbl. Bakt. (etc.), Abt. 1, Bd. 37, p. 169-177.
(22) KOCH, R.
1881. Zur Untersuchung von pathogenen Organismen. *In* Arb. K. Gsndhtsamt., Bd. 1, p. 1.
(23) KOPELOFF, N.
1916. The effect of soil reaction on ammonification by certain soil fungi. *In* Soil Sci., v. 1, no. 6, p. 541-573.
(24) LIPMAN, J. G.
1911. Bacteriological methods for estimating soil acidity. *In* Science, n. s., v. 33, no. 860, p. 971-973.

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(25) LIPMAN, J. G., and BROWN, P. E.
1908. Notes on methods and culture media. *In* N. J. Agr. Exp. Sta. Ann. Rpt. 1908, p. 129-136.
(26) LUCKHARDT, E.
1901. Ueber Variabilität und Bedingungen der Farbstoffbildung bei Spaltpilzen. *In* Dissertation, Freiburg. Abstract *in* Koch's Jahresber. Garungsorganismen., Bd. 12, p. 81-82.
(27) MACÉ, E.
1912-1913. Traite pratique de Bacteriologie, 2 vol., Paris, J. B. Bailliere.
(28) MARCHAL, E.
1893. The production of ammonia in the soil by microbes. *In* Bul. Acad. Sci. Belgique, (3), v. 25, p. 741.
(29) MIQUEL, P.
(a) 1879. Etude sur les poussières organisées. *In* Ann. Observ. Montsouris, p. 431-512.
(b) 1885. Organismes microscopiques de l'air et des eaux. *In* Ann. Observ. Montsouris, p. 568.
(30) MOORE, V. A.
1890. Preparation of nutritive agar. *In* Amer. Mo. Micros. Jour., v. 11, no. 5, p. 115.
(31) MOORE, V. A.
1912. Microbiology, p. 90. Carpenter and Co. Ithaca, N. Y.
(32) PETRI, R. J., and MAASSEN, A.
1892. Ueber die Bereitung der Nährbouillon für bakteriologische Zwecke. *In* Arb. K. Gsndhtsamt., Bd. 8, p. 30.
(33) PIERCE, N. B.
1900. Stock cultures and color preservation. *In* Jour. Appl. Micros. and Lab. Methods, v. 3, no. 3, p. 280-281.
(34) PRINGSHEIM, H., and PRINGSHEIM, E.
1910. Ueber die Verwendung von Agar-Agar als Energiequelle zur Assimilation des Luftstickstoffs. *In* Centbl. Bakt. (etc.), Abt. 2, Bd. 26, p. 227-231.
(35) PUCCINELLI, C.
1890. Il fucus crispus nella preparazione dei terreni nutritivi dei batteri. *In* Bul. Acad. Med. Roma, v. 16, fasc. 5, p. 247-257.
(36) RAVENEL, M. P.
1898. The preservation of culture media. *In* Jour. Appl. Micros. and Lab. Methods, v. 1, no. 6, p. 106.
(37) RICHTER, E.
1887. Agar agar for the cultivation of bacteria. *In* Berlin Klin. Wchnschr. (1887), p. 600.
(38) ROSENBACH, F. J.
1884. Mikro-organismen bei den Wundinfections-krankheiten des Menschen. 122 p. Wiesbaden, J. F. Bergmann.
(39) SCHOTTELIUS, M.
1887. Bereitung des Nähragar. *In* Centbl. Bakt. (etc.), Abt. 1, Bd. 2, p. 1042.
(40) SCHULTZ, N. K.
1891. Zur Frage der Bereitung einiger Nährsubstrate. *In* Centbl. Bakt. (etc.), Abt. 1, Bd. 10, p. 52.
(41) Report of the Committee on Standard Methods for the Examination of Water and Sewage. 1912. American Public Health Association.

Digitized by Google

(42) STEPHANDIS, PH.
1899. Ueber den Einfluss dess Nährstoffgehaltes von Nährböden auf die Raschheit der Sporenbildung. *In* Arch. Hyg., Bd. 35, p. 1-10.
(43) STERNBERG, G. M.
1892. A Manual of Bacteriology. 886 p. Wm. Wood and Co. New York.
(44) TEMPLE, J. C.
1911. The influence of stall manure upon the bacterial flora of the soil. Ga. Agr. Exp. Sta. Bul. 95, 35 p.
(45) THOM, C.
1910. The effects of acidity of culture media on morphology in species of *Penicillium*. *In* Science, n. s., v. 31, no. 799, p. 635.
(46) THOMANN, J.
1900. Ueber die Brauchbarkeit verscheidener Nährböden für die bakteriologische Wasseruntersuchung. *In* Centbl. Bakt. (etc.), Abt. 2, Bd. 6, p. 796-800.
(47) TRAAEN, A. E.
1915. Untersuchungen über Bodenpilze aus Norwegen. *In* Centbl. Agr. Chem., Bd. 44, no. 1, p. 347.
(48) WOLF, L.
1899. Ueber den Einfluss des Wassergehaltes der Nährböden auf das Wachsthum der Bakterien. *In* Arch. Hyg., Bd. 34, p. 200-209.
(49) WRIGHT, J. H.
1900. Studies on anærobic bacteria. *In* Jour. Boston Soc. Med. Sci., v. 5, no. 4, p. 114.

MISCELLANEOUS REFERENCES

(1) ALLEGER, W. W.
1899. Preparation of agar. *In* Jour. Roy. Micros. Soc., (1899), p. 543-544. See also Jour. Appl. Micros. and Lab. Methods, v. 1, (1898) no. 1, p. 8-9.
(2) BESSON, A.
1913. Practical Bacteriology, Microbiology and Serum Therapy (Medical and Veterinary): A Text Book for Laboratory Use. Translated and adapted from the Fifth French Edition, by H. J. Hutchens. 892 p. London, Longmans, Green and Co.
(3) BISSÉRIÉ, —.
1907. Procédé simple et rapids de preparation des milieux gélosés et gélatinés. *In* Ann. Inst. Pasteur, t. 21, p. 235-236.
(4) BLEISCH, M.
1895. Ein Apparat zur Gewinnung klaren Agars ohne Filtration. *In* Centbl. Bakt. (etc.), Abt. 1, Bd. 17, p. 360.
(5) BUCHNER, H.
1890. Effect of highly concentrated media on bacteria. *In* Centbl. Bakt. (etc.), Abt. 1, Bd. 8, p. 65-69.
(6) CHEESMAN, T. L.
1888. Preparation of nutrient gelatin and agar. *In* Amer. Nat., v. 22, p. 472-473.
(7) DRIGALSKI, V.
1906. Ein Schnellfilter für Agarlösungen. *In* Centbl. Bakt. (etc.), Abt. 1, Bd. 41, p. 298-301.

Digitized by Google

(8) EYRE, H.
1900. (a) Neutralization of media. *In* Brit. Med. Jour. (1900), p. 21.
(b) Nutrient media of "standard" reaction for bacteriological work. *In* British Med. Jour., (1900), p. 921.
(9) FISCHER, H.
1904. Ein einfaches Verfahren, Nähragar ohne Filtration zu klären. *In* Centbl. Bakt. (etc.), Abt. 1, Bd. 35, p. 527.
(10) FRAENKEL, A.
1886. Bakteriologische Mitteilungen. *In* Ztschr. Klin. Med., Bd. 10, p. 401-461.
(11) FREUDENREICH, E. VON,
1888. Die Bereitung des Nähragar. *In* Centbl. Bakt. (etc.), Abt. 1, Bd. 3, p. 797-798.
(12) GILTNER, W.
1916. A Laboratory Manual in General Microbiology. 418 p. John Wiley and Sons. New York.
(13) GAGE, S. M., and PHELPS, E. B.
1902. Untersuchungen von Nährboden zur quantitativen Schätzung von Bakterien in Wasser und Abwässern. *In* Centbl. Bakt. (etc.), 10.
Abt. 1, Bd. 32, p. 920-926.
(14) HAEGLER, S.
1895. Zur Agarbereitung. *In* Centbl. Bakt. (etc.), Abt. 1, Bd. 17, p. 558.
(15) HOLMAN, W. L.
1912. Rapid filtration of agar and gelatin. *In* Jour. Infect. Diseases, v. 10.
(16) KLUGE, —.
1896. Eine praktische Methode zur Herstellung von Agar für Kulturen. *In* Ztschr. Angew. Chem., Bd. 2, p. 237.
(17) LONDON, S.
1897. Schnelle und leichte Methode zur Bereitung des Nähragars. *In* Centbl. Bakt. (etc.), Abt. 1, Bd. 21, p. 686.
(18) VAN OEERBEEK DE MEIJER.
1891. Ueber die Bereitung des Nähragars. *In* Centbl. Bakt. (etc.), Abt. 1, Bd. 9, p. 163-165.
(19) NOVY, F. G.
1899. Nutrient culture media. *In* Jour. Appl. Micros. and Lab. Methods, v. 2, no. 1, p. 235-241.
(20) PAUL, T.
1901. Die Anwendung des Sandes zum schnellen Filtrieren des Nähragars. Abs. *in* Centbl. Bakt. (etc.), Abt. 1, Bd. 29, p. 270-271.
(21) PORODKO, T.
1908. The filtration of agar through glass-wool. Abs. *in* Jour. Royal Micros. Soc., (1909), p. 256.
(22) PRALL, F.
1902. Beitrag zur Kenntnis Nährböden für die Bestimmung der Keimzahl im Wasser. *In* Arb. K. Gsndhtsamt., Bd. 18, Heft 3, p. 436.
(23) REED, R. C.
1897. Preparation of culture media with special reference to sterilization. *In* Amer. Mo. Micros. Jour., v. 18, no. 5, p. 149.
(24) ROSANI, K.
1904. Beitrag zur Agarbereitung. *In* Centbl. Bakt. (etc.), Abt. 2, Bd. 12, p. 464.

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(25) RUZICKA, S.
1901. Zwei kleinere methodische Mitteilungen. *In* Centbl. Bakt. (etc.), Abt. 1, Bd. 29, p. 672-673.
(26) SCHULTZ, J. L.
1892. A rapid method of making agar agar. *In* Johns Hopkins Hosp. Bul. 24.
(27) TIMPE, H.
1893. Ueber den Einfluss der Eiweiss-Körper auf das Reaktion der Nährboden. *In* Centbl. Bakt. (etc.), Abt. 1, Bd. 14, p. 845.
(28) TISCHUTKIN, N.
1891. Eine vereinfachte Methode zur Bereitung von Fleisch-Pepton-Agar. *In* Centbl. Bakt. (etc.), Abt. 1, Bd. 9, p. 208.
(29) TUNMANN, O.
1909. Eine Bemerkung über Agar Agar. *In* Pharm. Centralhalle, Bd. 50, p. 233. Abs. *in* Centbl. Bakt. (etc.), Abt. 2, Bd. 27, p. 678-679.
(30) WALBAUM, H.
1901. Zur Methodik der bakteriologischen Wasseruntersuchung, mit Angaben über die Bereitung des Nähragars. *In* Centbl. Bakt. (etc.), Abt. 1, Bd. 30, p. 790-798.
(31) WOHL, M. G.
1913. A simple and rapid way of filtering agar agar. *In* Mo. Cycloped. and Med. Bul. Phila., v. 6, p. 29. Abs. *in* Centbl. Bakt. (etc.), Abt. 1, Bd. 58, p. 273-274.
(32) YOKOTE, T.
1899. Ueber die Darstellung des Nähragar. *In* Centbl. Bakt. (etc.), Abt. 1, Bd. 25, p. 379-380.
(33) ZUPNIK, L.
1895. Zur Agarbereitung. *In* Centbl. Bakt. (etc.), Abt. 1, Bd. 18, p. 202.

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PLATE III

Plates showing the effect of concentration of agar in media on bacterial counts, Soil IV.

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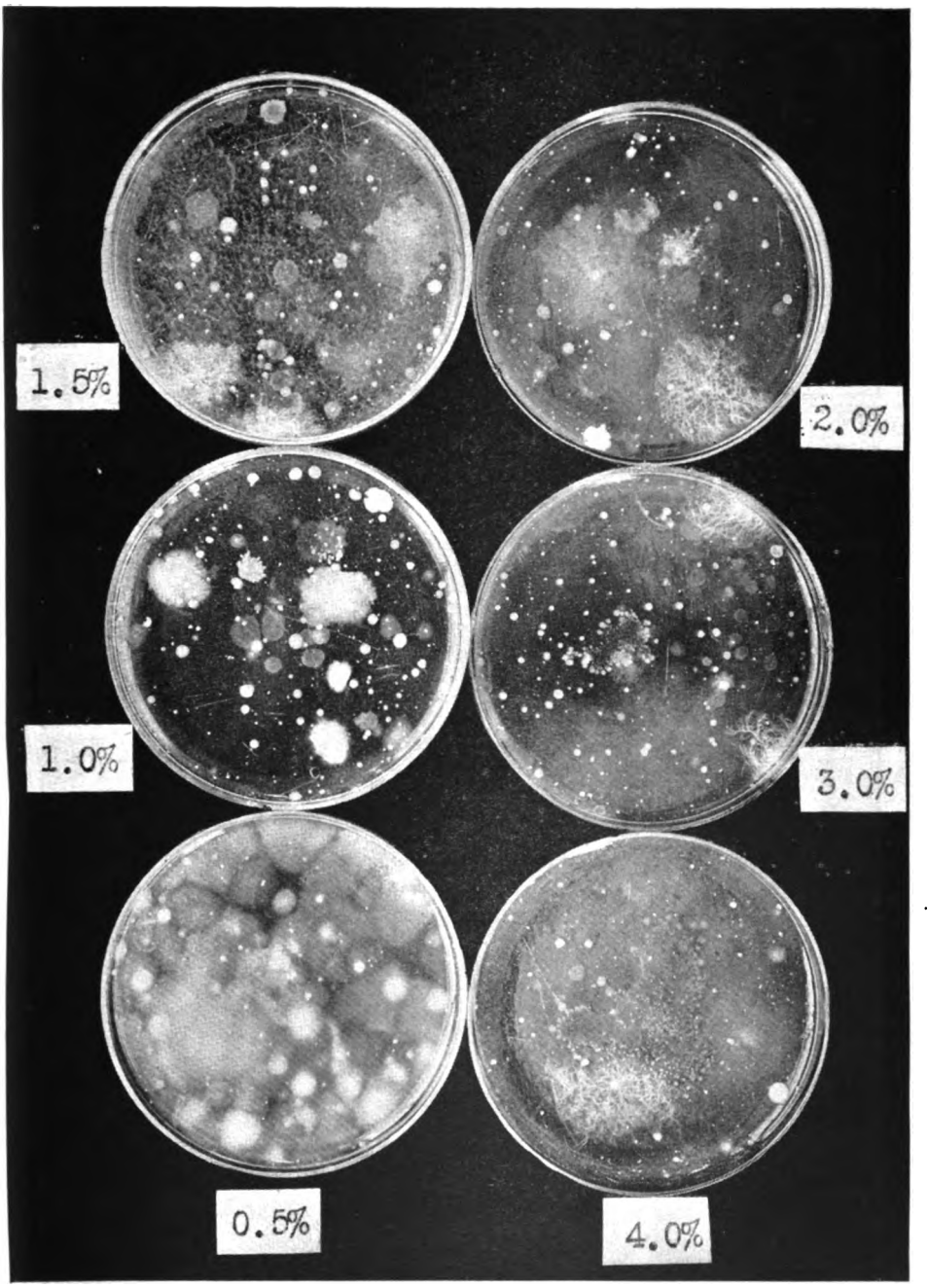

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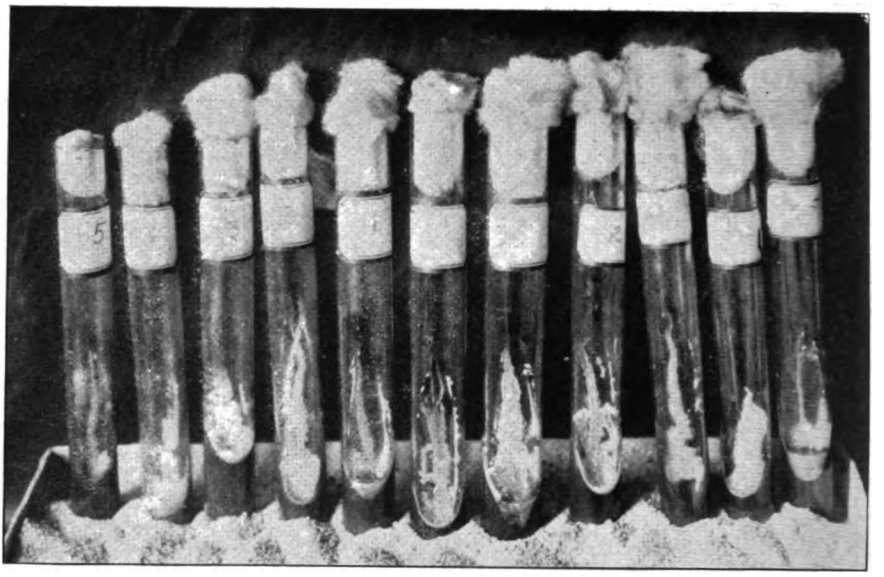

Fig. 1

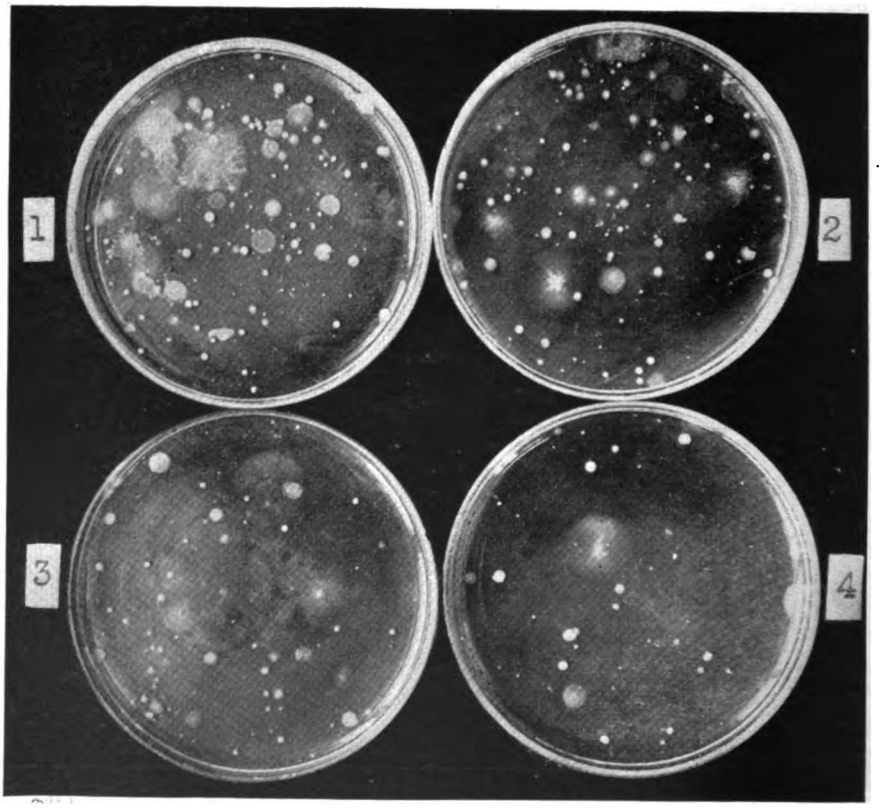

Fig. 2

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PLATE IV

Fig. 1.—Growth of *B. subtilis* on media of different reactions.
Fig. 2.—Plates showing the effect of sterilization of media at different pressures on bacterial counts, Soil I, Medium II, (Synthetic).
No. 1, Boiling Water.
No. 2, 1 Atmosphere.
No. 3, 2 Atmospheres.
No. 4, 3 Atmospheres.

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PLATE V

Plates showing the effect of sterilization of media at different pressures on bacterial counts. Fig. 1.—Water III, Medium I (Peptone).

No. 1, Boiling Water.
No. 2, 1 Atmosphere.
No. 3, 2 Atmospheres.
No. 4, 3 Atmospheres.

Fig. 2.—Water III, Medium II (Synthetic).

No. 1, Boiling Water.
No. 2, 1 Atmosphere.
No. 3, 2 Atmospheres.
No. 4, 3 Atmospheres.

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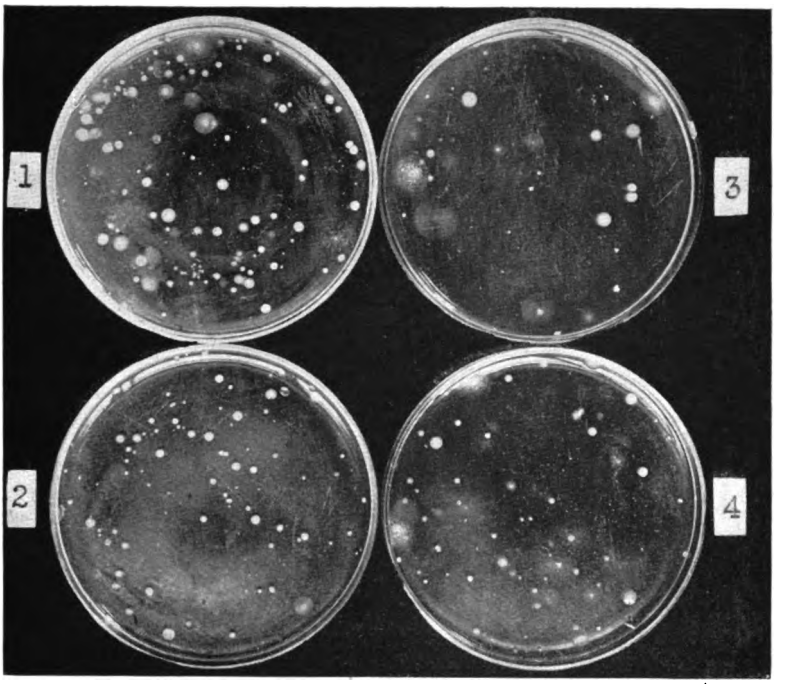

Fig. 1

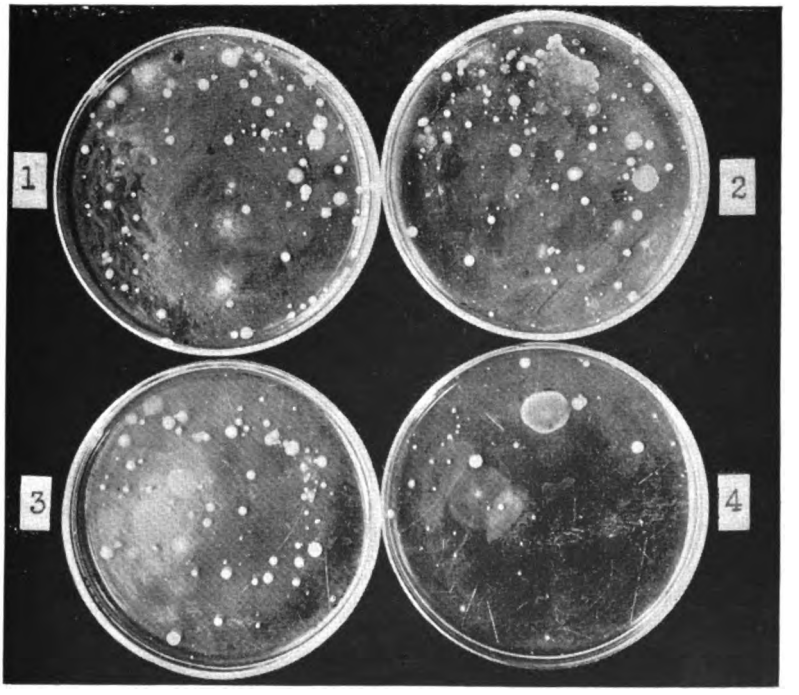

Fig. 2

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THE ORGANIC PHOSPHORUS OF SOIL[1]

By

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Introduction

That there must be phosphorus in organic combination in soil is universally recognized. It is shown in the historical section of this paper that there has been no method which can be said to give even an approximation as to the actual amount of organic phosphorus in soils. That such a method would be desirable is obvious. Phosphorus, from an economic standpoint, is perhaps the most important plant-food element in a consideration of the fertility of most of the soils of the United States. In many cases it is actually deficient in amount, and in many other cases, while present in fairly high proportions, it is in such condition that it is unavailable to plants. The presence of large amounts of organic matter in soils is known in many instances to change inert phosphates to such a condition that plants may utilize them. This is usually attributed to the increase in bacterial action due to the organic matter. As to just why this should make inert phosphates available there is some doubt, but the general opinion is that carbonic and other acids dissolve the phosphates. We would not venture to say whether or not this is the predominant action of the organic matter. It may be also that phosphorus actually taken up into the bacterial cells or into molds is of great importance, thus rendering available inert phosphates. It has been shown by Stoklasa (17), for instance, that some of the common soil bacterial cells contain about 1.5 per cent of phosphorus.

There is very little known as to the kinds of organic phosphorus compounds present in soil. However, from a knowledge of the organic materials added to soils one can draw some conclusions, but even here data are insufficient or unreliable. For instance, there is very little known in regard to the exact composition of vegetable material in organic phosphorus compounds. Phytin is known to be present to the extent of roughly 50 per cent of the phosphorus in the seeds of many grains, beans, etc. (5, p. 52), but as to the phosphorus in the stems and leaves very little is known. Practically nothing is known as to the quantitative relations of the phosphorus compounds in corn stover, oat straw, manures and such material which make up a large part of the organic matter ultimately finding its way to soil. The knowledge as to the quantitative composition of vegetable material in regard to its composition of nucleic

[1] Received for publication August 7, 1916.

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acid is negligible, yet since all cellular material contains nucleoproteins, this class of compounds must be added to soils in relatively large amounts.

The true lecithins are not found in plants (5, p. 73), and the phosphatids of the vegetable and animal kingdoms are essentially different. Not much is known in regard to plant phosphatids, but they seem to be widely but sparingly distributed. Because the large phosphatid molecule contains only one phosphorus atom, it is not probable that the phosphorus combined in phosphatids in soils is quantitatively of much importance.

Without going into the rather extensive literature on the chemical constitution of bacteria, molds and yeasts at this time, nucleoproteins, phosphatids and phytin are known to be present in many of them (16).

There is some doubt as to whether phosphoproteins are present in vegetable material at all. While some have apparently been isolated, yet it is claimed by many investigators that the phosphorus is present as an impurity (5, p. 48).

To generalize broadly from the rather meager data available, nucleoproteins and phytin probably make up most of the organic phosphorus compounds added to soil, with phosphatids next, and a very small quantity of phosphoproteins. As to the fate of the phosphorus compounds upon entering the soil, little can be said. So far as we know, nothing in regard to the action of soil bacteria upon phytin is known. It is known, however, to be very readily decomposed by enzymatic action (1). Stoklasa (17) has shown that nucleic acid is perhaps less readily attacked by bacteria than phosphatids. Casein, a phosphoprotein, is known to be readily decomposed upon being added to soil.

Historical

A brief review of the work done upon the organic phosphorus of the soil will be given here. For a quite complete bibliography on the subject up to the year 1909 the reader is referred to the article by Stewart (16).

Grandeau (9) believed that the phosphorus associated with the *matière noire* was in organic combination, and this view was upheld by other soil investigators. Others have opposed this idea. For instance, Van Bemmelen (18) believed that all of the phosphorus in the *matière noire* was simply absorbed. No direct or positive work tending to prove or disprove the question appeared until Fraps (6, 7) attacked the problem. He showed, amongst other things, that minerals, present in most soils, such at apatite, variscite, wavelite, vivianite, and many others, are somewhat soluble in 4 per cent ammonia. It was also found by him that ammonia dissolved considerable phosphorus from ignited soils and that the amount of phosphorus dissolved from ignited soils was increased by previous treatment with dilute acid. His conclusion was that the phosphorus dissolved by ammonia is certainly not all organic.

Stewart (16) determined the per cent of *matière noire* precipitated by 1 per cent hydrochloric acid and also the per cent of carbon, nitrogen

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and phosphorus so precipitated. These determinations were carried out on both untreated and 1 per cent acid extracted soil. From the fact that 238 pounds more phosphorus was obtained in the ammonia extract of the acid treated soil than in the untreated soil, Stewart concludes that all of this 238 pounds must be in organic combination; but Fraps' experiments showing that acid extracted ignited soils, necessarily containing no organic phosphorus, give more phosphorus into solution with ammonia than the unextracted ignited soils, render Stewart's conclusion, to say the least, not absolute. Since only 55 pounds of phosphorus was precipitated from the ammoniacal solution of the *matière noire* by acid and since less than 50 per cent of the carbon and nitrogen was so precipitated, Stewart concludes that there is considerable organic phosphorus in the filtrate. He is no doubt right, but as pointed out again by Fraps, his conclusion does not follow from the premises.

Stewart extracted his soil with cold 12 per cent hydrochloric acid. After washing free from chlorides, one portion was re-extracted with 12 per cent hydrochloric acid which dissolved 95 pounds of phosphôus per acre. Another portion was extracted with 4 per cent ammonia which dissolved 555 pounds of phosphorus per acre. The difference, 460, must represent, according to Stewart, organic phosphorus, but Fraps' finding that ammonia dissolves considerable phosphorus from minerals previously treated with 12 per cent hydrochloric acid makes this method of Stewart unreliable.

Schmoeger (13) found that ignition increased the phosphorus dissolved by 12 per cent hydrochloric acid, and from this fact assumed that the increased phosphorus dissolved after ignition was organic. In view of work already cited this can no longer be considered even approximately correct.

Hopkins and Pettit (10) proposed a calculation method to estimate the organic phosphorus of soil. In soils having the surface and subsoil with the same potassium content, the mineral composition of the two layers is assumed to be the same. Therefore, the excess of phosphorus in the surface soil is assumed to be organic. Such a method carries with it too many broad assumptions to be of much accuracy. Stewart found that fairly close agreement was obtained on one soil between this calculation method, the phosphorus dissolved by ammonia, the increase in phosphorus dissolved by 12 per cent hydrochloric acid after ignition of soil, and the increase of phosphorus dissolved after subjecting the soil to heat in the autoclave at 140° to 145° C. for 12 hours. The methods in general, however, have not been found to give concordant results. In view of the findings, particularly of Fraps, and also when we consider the assumptions necessarily involved in all of the methods, none of them can be considered to give accurate results.

There have been some successful attempts to isolate organic phosphorus compounds from the soil. Aso (2) extracted a soil with alcohol

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and ether and obtained a slight residue upon evaporation of the extract which was analyzed for phosphorus. According to the figures so obtained, he calculated his soil to contain 0.049 per cent lecithin. Stoklasa (17) also found indications of a small amount of lecithin by the same method. He was unable to isolate nucleic acid from soils. Shorey (14) isolated nucleic acid from a number of soils. No attempt was made by him to obtain quantitative results. From his observations, however, he states that nucleic acids "form a very appreciable portion of the organic matter of soils."

Experimental

The method which we have developed is not, for the present at least, proposed as giving the total organic phosphorus of soils, but rather as the total organic phosphorus in the dilute alkali extract of soils. This, of course, would be the lower limit for organic phosphorus. It has previously been shown in a publication from this laboratory that dilute sodium hydroxide extracts nucleic acid completely from soils (12). Nucleic acid is perhaps the most abundant organic phosphorus compound of soil and it may be that dilute alkali dissolves nearly all of the organic phosphorus. Confirmation of this will have to await further investigation.

The method which has been adopted after much experimentation is a combination of the Forbes (4) magnesia mixture method and the Emmet-Grindley (3) method, both slightly modified. The method in detail is as follows:

The soil extract, however obtained, is made slightly ammoniacal, sufficient ammonium chloride added to give a 5 to 10 per cent solution of the salt, and then magnesia added at the rate of 20 c.c. per 100 c.c. of solution. The magnesia mixture is added from a burette, a drop at a time, with constant stirring. The solution is allowed to stand 3 hours. The Forbes method calls for a much more strongly alkaline solution and a period of 12 hours of standing before filtration. It has been shown by Gooch and Austin (8) that the precipitation of a soluble phosphate is quantitative in the presence of a slight excess of ammonia if a sufficiently large excess of magnesia mixture is present. The reason for adopting the above procedure was to avoid as much hydrolysis of the organic phosphorus compounds as was possible. After standing the three hours, as much of the supernatant clear liquid was withdrawn as was possible. This was filtered. The residual liquid containing the large bulk of the precipitate was transferred to 100-c.c. tubes and centrifuged. The clear supernatant liquid was siphoned from the tubes and passed through the filter paper. The precipitate was washed four times with water containing a trace of magnesia mixture. After washing, the precipitate was collected with about 150 c.c. of water, and 8 c.c. of nitric acid of specific gravity of 1.20 was added for each 100 c.c. of the solution. The mixture was filtered and the residue washed with very dilute nitric acid. The

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filtrate and wash water was neutralized with ammonia and concentrated under diminished pressure to 125 c.c. The solution was then heated to 60° C. First, from 5 to 8 c.c. of nitric acid of specific gravity of 1.20, and then 100 c.c. of clear neutral ammonium molybdate were added. The temperature was maintained at 60° C. for 15 minutes and the solution stirred. The solution was then removed from the bath, and after standing about 2 hours the precipitate was filtered off and washed as usual. It was then dissolved in 200 c.c. of 2 per cent ammonia, 20 gm. of ammonium chloride added and 50 c.c. of magnesia mixture. After standing at least 3 hours the precipitate was filtered and washed in the usual manner and then dissolved in nitric acid and evaporated to complete dryness to remove silica. From this point the phosphorus was determined according to the method of Lorenz (11).

The total phosphorus of the soil was determined by the Lorenz method, the magnesium nitrate method of ignition being used. The total phosphorus of the various extracts was determined by the same method. In all cases the extracts were evaporated to dryness with magnesium nitrate solution and then ignited.

First of all, the proportion of the phosphorous extracted by N/5 acid in organic combination was determined. One hundred fifty grams of a silt loam soil were placed in each of 4 bottles and 600 c.c. of hydrochloric acid of such a strength that after contact with the soil it was N/5, were added to each bottle. After shaking 1 hour the mixtures were filtered. Two 400-c.c. portions of the clear filtrate were analyzed for total phosphorus and two 400-c.c. portions were analyzed for inorganic phosphates exactly as indicated above. The results follow:

TABLE I

COMPOSITION OF THE PHOSPHORUS IN THE N/5—HCl EXTRACT, EXPRESSED IN PER CENT OF SOIL

Total P in Soil	Total P in N/5—HCl Extract	Inorganic P in N/5—HCl Extract
0.0345	0.0112	0.01118

Therefore it is seen that all of the phosphorus extracted from this soil by dilute acid is inorganic in nature.

Two per cent ammonia was next experimented with. Because of the well-known fact that previous extraction of soils by dilute acids renders more of the organic matter soluble in dilute ammonia, and because of the fact that, as shown above, the dilute acid extracts no organic phosphorus, it was decided to extract the soil first with acid. The method used was the usual one, namely, placing about 150 gm. of the well ground soil on a Büchner funnel over filter paper and with gentle suction applied, about 1 per cent hydrochloric acid was poured over the soil in small portions.

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The soil was then washed thoroughly. In no case was the acid allowed to remain in contact with the soil for more than an hour.

After air-drying, the 2 per cent ammonia extract was made by shaking a mixture of 1 part of soil to 4 parts of alkali for 1 hour and then centrifuging for 5 minutes in the machine used for this work (15) in this laboratory. That 5 minutes' whirling in the machine takes out all phosphorus compounds in suspension is shown by the following experiment.

An alkali and soil mixture prepared in the usual way was placed in the centrifuge and after whirling 5 minutes, 50 c.c. were withdrawn and analyzed for total phosphorus. The whirling of the machine was continued for 10 minutes more and a second portion of 50 c.c. withdrawn and analyzed for total phosphorus. After whirling 45 minutes more, the same operation was repeated. The results are given in Table II.

TABLE II

RESULTS SHOWING THE EFFICIENCY OF THE CLARIFICATION OF THE EXTRACT

Time of Whirling in Minutes	Per cent of P in the Extract
5	0.01660
15	0.01657
60	0.01680

In order to test the method for the determination of the inorganic phosphorus in the alkali extract in regard to the completeness of the recovery of inorganic phosphorus, four portions of 200 c.c. of the 2 per cent ammonia extract were prepared in the usual way. To two of the solutions a known volume of a solution of potassium phosphate was added. All of the solutions were then carried through the procedure for the analysis of inorganic phosporus. At the same time the strength of the solution of potassium phosphate was determined by analyzing according to the Lorenz method. Several attempts were made before more than about 94 per cent of the added phosphorus could be determined. Finally by refining the procedure at various points by better washing, etc., a 98.9 per cent recovery was effected.

With the method thus developed, soil from a series of plots at the Experiment Station were analyzed. These plots are located on the Wisconsin drift soil area and are 1/20 acre in size. They have been fallowed since 1908. There are 14 in all, being numbered from 101 to 114. Not all were analyzed. In considering the results the topography of the plot must be taken into consideration. The highest point is near the south end of Plot 110. There is a gentle fan-like slope in all directions, the lowest part being in Plats 101 and 102. The results, together with the results on the soil used in the preliminary tests are given in Table III. This soil is from one of the orchards of the station and is considerably lower in organic matter than the soil from the plots.

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The results given in Table III are interesting. It is seen that the two plots receiving the more inert organic matter, namely 102 and 108, are lowest in organic phosphorus. If this is more than accidental, we are unable to assign any significance to the fact. All of the plot soils are higher in organic matter than the orchard soil and it is seen that all are considerably higher in organic phosphorus. Before any more definite conclusions are drawn the accumulation of more data must be awaited.

TABLE III

RESULTS OF THE PHOSPHORUS DETERMINATIONS
(Expressed as per cent of soil)

Soil	Treatment per Acre	Per cent P in Soil	Per cent P in NH_3 Extract	Per cent Inorganic P in NH_3 Extract	Per cent Organic P	Per cent P in NH_3 Extract	Per cent Inorganic P in NH_3 Extract	Per cent Organic P
Orch'd		0.0345	0.0166	0.0075	0.0091	48.1	21.7	26.4
102	2.8 T. Peat annually	0.0608	0.0383	0.0077	0.0206	63.0	12.7	33.9
103	8 T. Manure in 1909 and 1913	0.0451	0.0294	0.0119	0.0175	65.2	26.4	38.8
104	8 T. Clover in 1909 and 1913	0.0490	0.0303	0.0093	0.0210	61.5	19.0	42.9
107	Nothing	0.0459	0.0291	0.0114	0.0217	63.4	24.8	38.8
108	2 T. Oat Straw annually.....	0.0540	0.0327	0.0169	0.0158	60.6	31.3	29.1
111	4 T. Clover annually	0.0518	0.0318	0.0098	0.0220	61.4	18.9	41.2
114	4 T. Manure annually	0.0649	0.0377	0.0095	0.0282	58.1	14.7	43.5

Summary and Conclusions

The data obtained so far can only be regarded as preliminary. The work will be continued along the lines of an investigation into the completeness of the extraction of the organic phosphorus from the soil. Also, such questions as the influence of various factors on the change of organic phosphorus content of soil will be attacked. The availability of organic phosphorus compounds for plants under various treatments will be investigated.

While the method cannot be said to give an absolute knowledge of the organic phosphorus content of soil, it at least gives comparative results and should be of undoubted value in a comparison of various treatments on a single soil or closely related soils.

As a result of the work done so far, it can be definitely stated that a large part of the phosphorus of the soil is organic in nature.

Literature Cited

(1) Anderson, R. J.
1915. The hydrolysis of the organic phosphorus compound of wheat bran by the enzyme phytase. *In* Jour. Biol. Chem., v. 20, p. 483.

(2) Aso, K.
1904. On organic compounds of phosphoric acids in the soil. *In* Bul. Col. Agr., Tokyo Imp. Univ., v. 6, p. 277.

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(3) EMMETT, A. D., and GRINDLEY, H. S.
1906. A study of the phosphorus content of flesh. *In* Jour. Amer. Chem. Soc., v. 28, p. 25.
(4) FORBES, E. B., *et al.*
1910. Methods for the quantitative estimation of inorganic phosphorus in vegetable and animal substances. Ohio Agr. Exp. Sta. Bul. 215, p. 459-489.
(5) FORBES, E. B., and KEITH, M. H.
1914. Phosphorous compounds in animal metabolish. Ohio Agr. Exp. Sta. Bul. 5.
(6) FRAPS, G. S.
1908. The ammonia-soluble phosphoric acid of the soil. *In* Amer. Chem. Jour., v. 39, p. 579.
(7) FRAPS, G. S.
1911. Organic phosphoric acid of the soil. Tex. Agr. Exp. Sta. Bul. 136.
(8) GOOCH, F. A., and AUSTIN, M.
1897. Determination of phosphoric acid as magnesium pyrophosphate. *In* Amer. Jour. Sci., v. 4, p. 187.
(9) GRANDEAU, L.
1872. Recherches sur le rôle des matières organiques du sol dans les phénomènes de la nutrition des végètaux. *In* Compt. Rend. Acad. Sci. (Paris), t. 74, p. 988.
(10) HOPKINS, C. G., and PETTIT, J. H.
1908. The fertility of Illinois soils. Ill. Agr. Exp. Sta. Bul. 123.
(11) LORENZ, N.
1901. Phosphorsaurebestimmung in Dünger, Boden und Asche durch direkte Wägung des Ammoniumphosphomolybdes. *In* Landw. Vers. Stat., Bd. 55, p. 183.
(12) POTTER, R. S., and SNYDER, R. S.
1916. The soluble non-protein nitrogen of soil. *In* Jour. Agr. Research, v. 6, p. 61.
(13) SCHMOEGER, M.
1897. Sind die im Moor vorhandenen durch starke Sauren nicht extrahierbaren Phosphor — und Schwefel — verbindungen bereits in den Moorbildenden Pflanzen enthalten? *In* Landw. Jahrb., Bd. 26, 549.
(14) SHOREY, E. C.
1913. Some organic soil constituents. U. S. Dept. Agr. Bur. Soils Bul. 88.
(15) STEVENSON, W. H., WELLS, A. A., and COOVER, W. F.
1911. A centrifugal method for the determination of humus. Iowa Agr. Sta. Bul. 124.
(16) STEWART, ROBERT.
1910. Quantitative relationships of carbon phosphorus and nitrogen in soils. Ill. Agr. Exp. Sta. Bul. 145.
(17) STOKLASA, J.
1911. Biochemischer Kreislauf des Phosphat—Ions im Boden. *In* Centbl. Bakt. (etc.), Abt. 2, Bd. 29, p. 385.
(18) VAN BEMMELEN, J. M.
1890. Die Zusammensetzung der Ackererde nach Anleitung der in dem vorigen Abhandlungen mitgeleiten Analysen von gewöhnlichen und vulkarischen Thonböden. *In* Landw. Vers. Stat., Bd. 37, p. 347.

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DOES VANADIUM INTERFERE WITH THE DETERMINATION OF PHOSPHORUS IN SOILS WHEN THE PHOSPHORUS IS WEIGHED AS MAGNESIUM PYROPHOSPHATE?[1]

By

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In a recent paper, Robinson (8) gives the results of the following experiment: "Solutions containing 0.0050 gm. V_2O_5 and 0.00864 gm. P_2O_5 were treated with magnesia mixture in the usual manner and allowed to stand 6 hours; 0.0088 gm. P_2O_5 was found. When the precipitation for the same amounts had stood 17 hours there was apparently 0.0145 gm. P_2O_5. From these experiments it appears that with the small amounts of vanadium and phosphorous occurring in soils a satisfactory separation can be made with magnesia mixture, *provided the precipitate does not stand more than 3 to 4 hours.*"[2] Robinson does not suggest it, but the fact remains that this statement of his calls into question practically all of the determinations of phosphoric acid that have been made on either rocks or soils. Washington (9, p. 165) states that the magnesia precipitate should stand for 12 hours. Hilgard (3) recommends that the precipitate stand 24 hours before filtering. The Halle Experiment Station (10) allows 48 hours for the complete separation of the precipitate.

[2] Our italics.

This list might be continued almost indefinitely, for at least 50 per cent of the texts on quantitative analysis specify that it is desirable that the precipitate stand "over night" and, even in those texts which state that 3 to 4 hours' standing causes complete precipitation, there is no hint that a longer standing may produce erroneous results.

Inasmuch as vanadium is probably present in most soils (7) and rocks (4) in amounts which in some instances equal or even exceed the phosphorous content, it was deemed advisable to see if Robinson's conclusion as to the possible contamination of the magnesium pyro-phosphate by vanadium was actually justifiable.

[1] Received for publication June 13, 1916.

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A contamination of the magnesium precipitate by vanadium would occur only providing the vanadium was precipitated both by the molybdate and by the magnesia mixture. It is well established that vanadium is precipitated by ammonium molybdate only in the presence of phosphoric acid, and that *complete* precipitation of the vanadium requires a large excess of phosphorous. Cain and Hostetter (1, p. 254) state that 10 mg. of phosphorous is necessary for the complete precipitation of 1 mg. of vanadium.

It would seem, therefore, that in a soil analysis a separation of phosphorous and vanadium would be made, to a large extent at least, in the molybdate precipitation, and that sufficient quantities of vanadium would not remain to contaminate seriously the magnesia precipitate, even if all of the remaining vanadium should be precipitated by the magnesia mixture. On this latter point, Robinson's experiment furnishes the only available data that we have been able to find.

We have, therefore, investigated the behavior of vanadium when added directly to a phosphate solution and precipitated by magnesia mixture as well as when added to soil extracts and precipitated by ammonium molybdate followed by the usual magnesia precipitation.

Experimental

Precipitation of a mixed solution of phosphate and vanadate by magnesia mixture

It will be noted in Robinson's experiment that at the end of 6 hours the P_2O_5 recovered was but 0.00014 gm. in excess of that added, while at the end of 17 hours it was 0.00586 gm. in excess, or slightly more than would be produced by the complete precipitation of the added vanadium. Such a differential rate of precipitation is certainly unusual, and if *complete* precipitation of vanadium occurs in 17 hours it should take more than a single determination to show that only a mere trace is precipitated in 6 hours.

It was suggested to us that a possible explanation of the vanadium precipitation might be found in the well known fact that a high concentration of ammonium salts causes ammonium meta-vanadate to precipitate from solution. We have, therefore, tested the behavior of a mixture of sodium phosphate and ammonium meta-vanadate when treated with magnesia mixture in the presence of varying concentrations of ammonium hydroxide. The volume of the solution in which the precipitation was made was, in each instance, 100 c.c. The data are shown in Table I.

It will be observed that an increase in the weight of the magnesium precipitate was obtained only when the concentration of the ammonia exceeded 20 per cent by volume. In all instances where vanadium was added, the magnesium pyrophosphate was more or less colored, ranging

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from light yellow to almost orange. However, the amount of color present did not correspond accurately to increases in weight.

These experiments would indicate that, even if all of the vanadium of soils were present in the ammonium phospho-molybdate precipitate, a contamination of the pyrophosphate precipitate would not normally take place, inasmuch as the concentration of ammonia in the solution should not exceed 20 per cent by volume. There is a possibility, however, that

TABLE I

THE WEIGHTS OF MAGNESIUM PYROPHOSPHATE OBTAINED BY PRECIPITATING PHOSPHORIC ACID WITH MAGNESIA MIXTURE IN THE PRESENCE OF DIFFERENT AMOUNTS OF AMMONIUM META-VANADATE AND VARYING CONCENTRATIONS OF AMMONIA, THE PRECIPITATE IN ALL CASES STANDING 20 HOURS

P_2O_5 Taken gm.	V_2O_5 Taken gm.	$Mg_2P_2O_7$ Found gm.	$Mg_2P_2O_7$ Found gm.	$Mg_2P_2O_7$ Found gm.
		10%[1] NH_4OH	20%[1] NH_4OH	30%[1] NH_4OH
0.0032	None	0.0049	0.0051	0.0050
0.0032	None	0.0050	0.0048	0.0051
0.0032	0.0014	0.0050	0.0049	0.0055
0.0032	0.0014	0.0050	0.0052	0.0061
0.0032	0.0028	0.0050	0.0050	0.0058
0.0032	0.0028	0.0050	0.0051	0.0066
0.0032	0.0042	0.0051	.....	.....
0.0032	0.0042	0.0051	.....	.....

[1] Per cent by volume of concentrated ammonia.

the vanadium might be precipitated from solution by some conditions which did not occur in our experiments, and we have, therefore, tested the effect of vanadium on the determination of the phosphorous contained in a soil solution.

The effect of vanadium on the determination of soil phosphorous

A standard solution of ammonium meta-vanadate was prepared and added in different amounts to a soil solution prepared by a 5-day digestion of a soil with 1.115 specific gravity hydrochloric acid and subsequent procedure as for Solution "A" in Hilgard's (3) manual. The hydrochloric acid solution after the addition of the vanadium was evaporated with nitric acid to change chlorides into nitrates, taken up with dilute nitric acid and the phosphoric acid precipitated with ammonium molybdate and ammonium nitrate in the usual manner. In all cases the molybdate precipitate was left in a warm place over night. The influence of the vanadium could be readily observed, since where vanadium was added the precipitate was colored more or less deeply orange. The magnesium ammonium phosphate was precipitated as usual, in an ammonia concentration of 15 to 18 per cent by volume, and after standing for 3 hours was filtered off and weighed. The filtrates were then allowed to stand 24 hours longer in order to determine how much V_2O_5 would be precipi-

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tated between 3 hours and 27 hours. According to Robinson's data, all of the vanadium precipitated by the ammonium phospho-molybdate should have appeared here. Table II shows the results obtained.

TABLE II

RESULTS SHOWING THE EFFECT OF VANADIUM UPON THE DETERMINATION OF SOIL PHOSPHOROUS, MAGNESIUM PRECIPITATE REMOVED AFTER 3 HOURS

P_2O_5 Taken gm.	V_2O_5 Added gm.	$Mg_2P_2O_7$, 3 hrs. gm.	P_2O_5, 3 hrs. gm.	$Mg_2P_2O_7$ in filtrate, 3 to 27 hrs. gm.
None	0.0028	None	None	0.0001
None	0.0028	0.0002	.....	0.0002
0.0026	None	0.0044	0.0028	0.0001
0.0026	None	0.0041	0.0026	None
0.0026	None	0.0043	0.0027	0.0001
0.0026	0.0014	0.0037	0.0024	.....
0.0026	0.0014	0.0041	0.0026	.....
0.0026	0.0028	0.0037	0.0024	0.0001
0.0026	0.0028	0.0039	0.0025	0.0002
0.0026	0.0056	0.0039	0.0025	0.0002
0.0026	0.0056	0.0040	0.0026	0.0003

It will be noted that the added vanadium has in no way interfered with the determination of phosphorous and that the vanadium was not precipitated from the filtrate after filtering off the magnesium ammonium phosphate.

Thinking that perhaps the vanadium might be precipitated only in the presence of the magnesium ammonium phosphate, the experiment was repeated, the magnesium precipitate being allowed to stand 24 hours. The results are given in Table III.

TABLE III

RESULTS SHOWING THE EFFECT OF VANADIUM UPON THE DETERMINATION OF SOIL PHOSPHOROUS, MAGNESIUM PRECIPITATE ALLOWED TO REMAIN 24 HOURS

P_2O_5 Taken gm.	V_2O_5 Added gm.	$Mg_2P_2O_7$, 24 hrs. gm.	P_2O_5, 24 hrs. gm.
0.0043	None	0.0066	0.0042
0.0043	None	0.0069	0.0044
0.0043	0.0014	0.0067	0.0043
0.0043	0.0014	0.0071	0.0045
0.0043	0.0028	0.0067	0.0043
0.0043	0.0028	0.0067	0.0043

It will be noted that the results are not altered by the added vanadium. The ammonium phospho-molybdate was quite deeply colored in those experiments where vanadium was added, but the tests of the filtrates from the magnesium precipitate showed that the vanadium was still in solution in the ammonia. The ignited precipitates had only a faintly yellow tint and showed only traces of vanadium.

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To make our position absolutely sure, however, a third series of experiments was undertaken. In this instance the phosphoric acid was determined not only gravimetrically as pyrophosphate, but the yellow precipitate was also weighed on a Gooch crucible as $(NH_4)_3PO_4 \cdot 12\ MoO_3$ after drying at 160° to 180° to constant weight (5, p. 598), and also determined volumetrically according to the method of J. A. Prescott (6). The results are shown in Table IV.

TABLE IV

RESULTS SHOWING THE EFFECT OF VANADIUM UPON THE DETERMINATION OF SOIL PHOSPHOROUS BY THREE DIFFERENT METHODS

P_2O_5 Taken gm.	V_2O_5 Taken gm.	P_2O_5 as $Mg_2P_2O_7$ after 24 hrs. gm.	P_2O_5 as Yellow Ppt. Grav. gm.	P_2O_5 Titration of Yellow Ppt. gm.
0.0038	None	0.0039	0.0037	0.0038
0.0038	None	0.0038	0.0036	0.0038
0.0038	None	0.0036	.....	0.0038
0.0038	0.0014	0.0042	0.0036	0.0038
0.0038	0.0014	0.0040	0.0036	0.0037
0.0038	0.0028	0.0037	0.0036	0.0036
0.0038	0.0028	0.0037	0.0036	0.0036

There is no doubt that some vanadium does come down with the yellow precipitate, but that the amount which does precipitate is only a small part of that in solution was demonstrated by qualitative tests on the filtrate from the molybdate precipitation (cf. Cain and Hostetter, Reference 1).

It is equally well demonstrated that when vanadium is present, the precipitation of phosphorous by ammonium molybdate is interfered with and may be incomplete (2). We have purposely limited ourselves to the phosphorous content present in normal soils, using only soil solutions in order to have all of the usual elements present in their usual quantities, and adding vanadium in such quantity as to considerably exceed the maximum amount which would probably be expected in soils.

Possibly our final results represent, to a slight extent, a balancing of errors, but we believe that we have demonstrated that the earlier determinations of phosphoric acid in rocks and soils do not contain appreciable errors due to the presence of vanadium, and that the vanadium present in the soil will not interfere with either the volumetric or the gravimetric determination of phosphorous, even though the magnesia precipitate should stand for 24 hours.

Summary

Robinson suggests that vanadium, if present in a soil or rock, may be precipitated together with the phosphoric acid by ammonium molybdate and may introduce grave errors in the determination of phosphorous as magnesium pyrophosphate if the magnesium precipitate is allowed to

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stand more than 3 to 4 hours. This statement is apparently based upon a single determination.

We have made a series of determinations, using soil solutions to which known amounts of vanadium were added, the phosphorous being weighed as pyrophosphate, after standing for 24 hours, and in no instance do we find any appreciable interference by vanadium. The titrametric method for phosphorous is also unaffected by the presence of vanadium in amounts far in excess of the usual soil content.

Literature Cited

(1) Cain, J. R., and Hostetter, J. C.
1912. A rapid method for the determination of vanadium in steels, ores, etc., based on its quantitative inclusion by the phospho-molybdate precipitate. *In* Jour. Indus. Engin. Chem., v. 4, p. 250.

(2) Cain, J. R., and Tucker, F. H.
1913. Determination of phosphorous in steels containing vanadium. *In* Jour. Indus. Engin. Chem., v. 5, p. 647-650.

(3) Hilgard, E. W.
1903. Methods of physical and chemical soil analysis. Univ. Cal. Agr. Exp. Sta. Circ. 6.

(4) Hillebrand, W. F.
1910. The analysis of silicate and carbonate rocks. U. S. Geol. Survey Bul. 422.

(5) Mellor, J. W.
1913. A Treatise on Quantitative Inorganic Analysis. J. B. Lippincott Co., Philadelphia.

(6) Prescott, J. A.
1914. The estimation of phosphates in soil extracts. *In* Jour. Agr. Sci., v. 6, p. 120.

(7) Robinson, W. O.
1914. The inorganic composition of some important American soils. U. S. Dept. Agr. Bul. 122.

(8) Robinson, W. O.
1916. A comparison of methods for the determination of soil phosphorous. *In* Jour. Indus. Engin. Chem., v. 8, p. 148-151.

(9) Washington, H. S.
1910. The Chemical Analysis of Rocks. John Wiley and Sons, New York.

(10) Wiley, H. W.
1906. Principles and Practice of Agricultural Analysis: Soils. v. 1, p. 254-255. Chemical Publishing Co., Easton, Pa.

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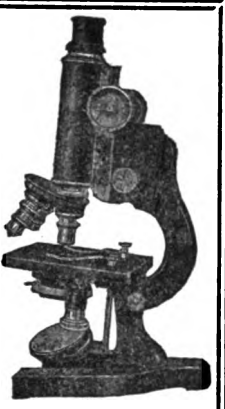

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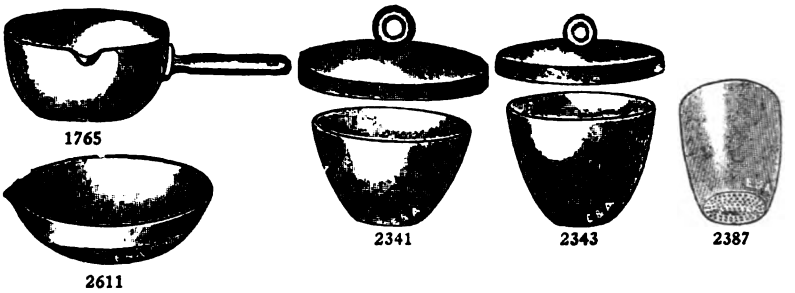

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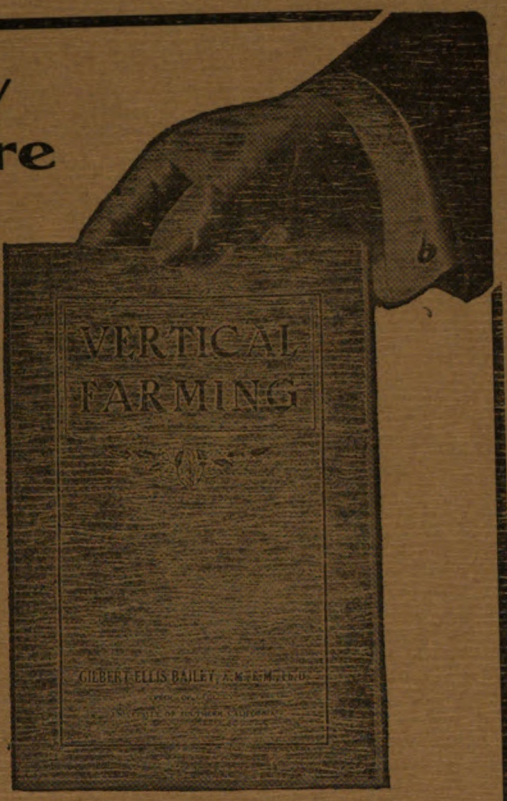

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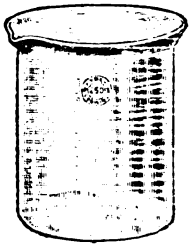

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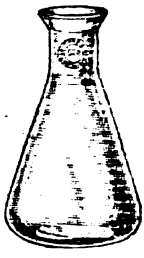

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SOIL SCIENCE

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Volume II **Number 4**

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SOIL SCIENCE

RUTGERS COLLEGE

VOL. II NEW BRUNSWICK, N. J., OCTOBER, 1916 No. 4

FACTORS AFFECTING THE ABSORPTION[1] AND DISTRIBUTION OF AMMONIA APPLIED TO SOILS[2]

BY

R. C. COOK, *Research Fellow, Rutgers College*

INTRODUCTION

As early as the sixteenth century it had been observed that coastal sands possessed the property of extracting salts from sea water. Since that time the purification of water by similar means has become a matter of such common observation that now it is hardly given a thought. And just as sands are able to retain dissolved matter, so soils in general act to a much larger extent as an immense reservoir in conserving plant-food that would otherwise be lost. It is therefore possible that many of the soil processes owe their effectiveness largely to this property of salt retention. The holding of soluble potash, phosphoric acid and nitrogen in soils against the leaching action of rains is a matter of economic importance and one that cannot well be overlooked. In fact, absorption by soils has been correlated with their crop-producing power and does undoubtedly bear some direct relation to soil fertility.

That this phenomenon is the resultant of numerous forces, and therefore very complicated, can hardly be disputed in view of the extensive, and often conflicting, observations which have been reported. In general, it has been the tendency to attribute the facts observed as due to simple causes acting independently of one another. Such factors as mechanical and chemical composition of soils, as well as hygroscopic moisture and colloids, some entirely chemical, others entirely physical, have been pointed out as possible explanations. Undoubtedly some, if not all of these, exert their influences, but from a study of the work following it seems clear that no one or two of these elements are wholly responsible.

[1] By the term "absorption" is meant the withdrawal from solution, without regard to whether it is due to physical, chemical or other causes.

[2] A thesis submitted to the faculty of Rutgers College in partial fulfillment of the requirements for the degree of Master of Science in Research, 1916.

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It has been suggested that absorption could be due to one, or more, of three causes: first, physical absorption; second, chemical absorption in the formation of an insoluble compound; or third, the "concentration of the dissolved substance on or about the surface of the absorbing medium" (5). The problem appears to be one for the physical chemist and will probably owe its complete solution to the full development of that field of science. At best, the work done at present must be largely superficial but may be justified as a contribution to the facts already known.

Broadly speaking, soil absorption manifests itself somewhat differently toward different materials. The absorption of phosphoric acid is undoubtedly controlled by factors different from those controlling that of potash or ammonia, while all three may have elements more or less in common. The work herein reported is limited to a study of that phase of absorption relating only to ammonia. In outlining the problem, it has been the desire of the writer to add something not only of scientific interest but also of practical significance in the use of ammonium sulfate as a fertilizer.

Historical

We are probably indebted to Way (30) for the first systematic researches upon the subject. He observed that when a solution of a salt was placed in contact with soil, a portion of the base, but none of the acid, was removed. In trying to determine which of the soil constituents was responsible, he passed a solution of ammonia through pure sand and found no change took place with this material. He then oxidized the organic matter of the soil with nitric acid and treated it with ammonia as before. Since no ammonia passed through the soil, he concluded that neither the organic matter nor the sand was essential to absorption. After numerous other tests he showed that with the soils used, clay was the main element causing the absorption phenomena. In attempting to trace out the particular compound which caused the absorption in the clay, he succeeded in producing a hydrated silicate of aluminum and soda which manifested absorptive properties similar to those of the soil. The addition of chalk to the soil in no way influenced the absorption of ammonia. Knop (15) confirmed this observation regarding lime.

It was shown by Eichorn (8) that natural hydrated silicates had the same power as Way's artificial preparation. Biedermann (3), Rautenberg (22) and Heiden (12) showed that the absorptive power bears a relation to the amount of soluble silicates contained. Tuxen (28) observed that the presence of salts of soda and potash in solution decreases the power of a salt to absorb ammonia. That hydrated oxides of iron and alumnia are capable of absorbing potash and ammonia was shown by Warington (29).

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Armsby (2) points out that the absorption is accompanied by a chemical reaction between the salt whose base is absorbed and some constituent of the soil. He concludes from his own work and that of others that:

"1. The absorption of combined bases by the soil consists in an interchange of base between the salt and the hydrous silicates of the soil.

"2. This exchange, which is primarily chemical, is only partial, its extent varying

"(a) With the concentration of the solution, and

"(b) With the ratio between the volume of the solution and the quantity of the soil used.

"3. The cause of these variations is probably the action of mass or the tendency of the resulting compounds to reform the original bodies, the absorption actually found in any case marking the point where the two forces are in equilibrium."

Wiley (34) states that "it is now generally accepted that the absorption of salts of the alkalies, accompanied by the change of base, is due chiefly to the presence of decomposed zeolite minerals in the soil. Besides the purely chemical absorption of salts by the soil, we have a physical absorption of various substances similar to the action of charcoal when used as a filter."

Whitney (31) believes that the surface area of soils is largely responsible for their absorption properties. Huston (13) has calculated that an acre nine inches of soil will fix ammonia equivalent to more than 16,000 pounds of sulfate of ammonia, and that the problem is therefore of no practical significance.

Dittrich (7) shows that the absorption of ammonia is similar to that of potash, and that in both cases the union seems to be a chemical one.

In investigating the absorptive capacity of the different soil layers, Karpizov (14) found it at a maximum in the surface layer, decreasing directly with the humus and zeolites toward the deeper subsoil. The degree of absorption varied with the hygroscopic moisture content, carbonates, and to some extent with mechanical composition. Sabanin (25) confirms the observations of the relation of hygroscopic moisture and mechanical composition.

In certain Russian soils Zholtsinski (35) found also that hygroscopic moisture, mechanical composition and ammonia absorption bore a relationship to one another. The bulk of absorption was in the finest particles below .01 mm. diameter.

The presence of lime concretions was shown by Blanck (4) to have a marked effect upon the absorption in soils. Hall and Gimingham (11) found that when ammonium salts were in excess, a given weight of clay removed the same amount of ammonium from solution whatever the strength of the latter, if given time. Different constituent materials

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of the soil, sand, clay, humus, and calcium carbonate, were tried for absorption. Sand gave negative results, while clay seemed mainly responsible. Humic acids were thought to form some insoluble compounds with ammonia. Calcium carbonate increased the absorption slightly.

Patten and Waggaman (20), having studied the subject of absorption very carefully, worked out a number of points in this connection. The mechanism of the process is studied and several formulae are derived to express certain functions, such as rate and extent of absorption in soils. The work is largely complementary to that referred to in the foregoing pages.

Morse and Curry (19) observed that clays react with potassium, sodium and ammonium in equivalent quantities. Calcium, magnesium, iron and aluminum constitute the largest amount of reacting bases. The extent of the reaction is dependent upon the concentration of the salt solution.

Rohland (23) and Wiegner (33) do not think the absorption chemical, but are inclined toward explaining it by a colloidal-physical theory. Pratolongo (21) divides the compounds of soils into solid solutions and absorptive compounds and uses this division as an aid in explaining the observed facts. The relation between the adsorption by soils and their hygroscopicity is shown again by Aberson (1). His experiments also indicated that "an equilibrium was established in the exchange of ions of the soil and of the added solution. The addition of ions which reacted with those in the solution reduced the adsorption. Soil adsorption followed the same law as adsorption by charcoal, wool, silk, etc. Adsorption in the soil is held to be a function of the surfaces of the colloidal substances."

Stoklasa (26) studied absorption in sterilized and unsterilized soils. Finding a difference in favor of the unsterilized soils, he concluded that there was a biological factor which may supplement those of a chemical or physical nature.

Lemmermann and Fresenius (16) worked chiefly with the volatilization of ammonia in soils, finding that $CaCO_3$ increased the absorption, while CaO had the opposite effect. KCl, NaCl, and sulfates and carbonates of these bases, as well as kainit and Thomas slag, reduced the absorptive power.

Wiegner (32) studied the exchange of bases when he used molecular equivalents of silicate gels, containing SiO_2, Al_2O_3, CaO, K_2O and H_2O. The absorption of ammonia liberated equivalent amounts of Ca and K ions. The influence of temperature was small; raising it caused a slight decrease in absorption. The degree of fineness of the silicate had but little effect upon absorption. Dehydration lessened it, at first only a little, but later reduced it to almost nothing.

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Measurements of the absorption of ammonia on five soil types by Engles (9), at periods of 4 hours, 1, 8 and 14 days, showed practically no further increase after 4 hours. Absorption increased with lime, iron and alumina contents of the soils. Mitscherlich (18), after a thorough study of absorption by soils, concludes that solution and absorption constitute a cyclic process. Whether one or the other takes place will depend wholly upon the conditions present.

Crawley and Duncan (6) in attempting to locate the depth of soil which was responsible for the fixation of ammonia found that one-half of it was fixed in the first inch, four-fifths in the first two inches, and almost all in the first four inches.

McGeorge (17) found again that the absorption of ammonia was parallel to that of potash and corresponds in part to the amount of lime and magnesia present.

Gans (10) opposes the view of Wiegner that the phenomena of absorption are due to physical causes, and believes they are chiefly due to chemical ones instead. His beliefs are based upon the fact that aluminum silicate always absorbs the same amount of ammonia regardless of the concentration of the solution.

Tadskaro (27), in studying acid soils of Japan, finds that while the absorption of coloring matter follows very well the expansion and hygroscopic power (as functions of colloidal materials, supposedly), the absorption of ammonia does not. He believes that the fixation of ammonia represents more complicated conditions. Of the soils studied, the humus soils absorbed most ammonia, the clay soils the next largest amount, and the sandy soils least.

Ruprecht and Morse (24) show again that increase in concentration of the solution increases the absorption of ammonia, and that between limed and unlimed soils the former usually absorb the larger amount. They state that the absorption of ammonia is very similar to that of dyes, and believe that "the ammonia is held by physical rather than by chemical forces."

In reviewing the researches concerning this subject one cannot help being impressed by the extremely varied and conflicting observations reported. There appears to be ample evidence that the problem is a very complex one and that perhaps the apparent contradictions are only the result of different combinations of factors.

It is doubtful if any limited amount of work will wholly explain the mechanism of absorption of ammonia by soils, and the following is merely a contribution which may help somewhat in our understanding of it.

Purpose

In working upon the problem of ammonia fixation it was purposed to find, if possible, any additional information concerning the factors which

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are responsible, as well as to learn more of what controls them. The application to several typical soil types of New Jersey was to be studied in particular, with its accompanying problems.

Considerable work has been done in connection with the method of application of ammonium sulfate to soils, with the result that many conflicting practices and theories have been brought out. A study of the vertical distribution of ammonia when applied to soils appeared to offer an opportunity of shedding some light upon this particular point.

Plan

Samples of soil of widely differing character representing six prominent soil types of New Jersey were obtained. They are designated respectively by the Bureau of Soils of the U. S. Department of Agriculture, as Norfolk sand, Collington sandy loam, Sassafras loam, Dutchess silt loam, Penn loam, and Alloway clay.

The Norfolk sand, typical of a large area of coastal plains soils, is a very light soil low in organic matter. The Collington sandy loam was one which had been recently limed and is distinctly alkaline in reaction. It consists of moderately coarse sand and greensand marl with very little fine material. The Sassafras is a fairly heavy fertile loam. The Dutchess silt loam is a very heavy soil resembling the Penn loam in physical properties. The Alloway clay is a very stiff soil but is not as heavy as either the Dutchess silt loam or the Penn loam. As ordinarily classified the soils would be placed in the order suggested in the tables, but perhaps if arranged from lightest to heaviest, as indicted by the moisture-holding capacity, the order would rather be: Norfolk sand, Collington sandy loam, Alloway clay, Sassafras loam, Dutchess silt loam, and Penn loam.

Experimental

Part I

Absorption of Ammonia in Different Soil Types and some Factors Affecting It

Several methods have been devised for the determination of the absorptive capacity of soils, but so far the simple method used by Way in 1850 seems to be as satisfactory as any suggested. This method consists in the shaking of a given weight of soil in a flask with a given volume of a solution of known strength, allowing the mixture to stand until an equilibrium is reached and determining the concentration of the resultant solution. By difference in the solution strength the absorption is obtained. The objection has been raised to this method that it does not in any way approximate natural conditions and therefore the results cannot be applied. However true this may be, the other methods do not appear to possess any particular advantage.

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But for the work in hand a comparison was made in order to see which one would be best suited for the purpose. The first consisted in shaking 50 gm. of soil for one minute with 200 c.c. of a 1/100 normal solution of sulfate of ammonia (34 mg. of ammonia), allowing to stand for a period and distilling off the ammonia from a filtered aliquot by the use of NaOH. The time of standing exerted some influence, as can be seen by referring to Table I. But the increase in absorption after 4 hours

TABLE I

ABSORPTION OF AMMONIA BY SOILS AS AFFECTED BY TIME[1]

Soil No.	1 Hour NH_3 Absor'd		2 Hours NH_3 Absor'd		4 Hours NH_3 Absor'd		24 Hours NH_3 Absor'd		48 Hours NH_3 Absor'd		72 Hours NH_3 Absor'd	
	Mg.	Av. Mg.	Mg.	Av. Mg.	Mg.	Av. Mg.	Mg.	Av. Mg.	Mg.	Av. Mg.	Mg.	Av. Mg.
I	1.0	.....	1.0	.....	1.4	.....	1.0	.....	1.5	.....	[2]0.34	.....
	1.0	1.00	1.4	1.20	1.4	1.40	1.4	1.2	1.4	1.45	1.40	1.40
II	11.9	.....	12.6	.....	14.6	.....	14.6	.....	14.3	.....	14.90	.....
	11.2	11.55	11.6	12.10	13.9	14.25	13.9	14.25	14.6	14.45	14.60	14.75
III	8.2	.....	8.5	.....	9.2	.....	9.2	.....	9.2	.....	10.20	.....
	8.2	8.20	8.0	8.25	9.4	9.30	9.5	9.35	9.7	9.45	10.20	10.20
IV	5.4	.....	6.5	.....	7.8	.....	9.0	.....	8.8	.....	9.20	.....
	6.5	5.95	7.1	6.80	8.2	8.00	9.0	9.00	8.8	8.80	9.20	9.20
V	8.7	.....	9.9	.....	10.9	.....	11.0	.....	11.9	.....	11.90	.....
	8.3	8.50	9.0	9.45	10.9	10.90	11.7	10.35	11.4	11.65	12.60	12.75
VI	6.1	.....	6.1	.....	6.6	.....	6.5	.....	7.3	.....	7.80	.....
	5.4	5.75	6.5	6.30	6.3	6.50	6.5	6.50	7.1	7.20	7.51	7.65

[1] 200 c.c. of N/100 NH_3 solution added to 50-gm. portions of soil, shaken at intervals, and NH_3 determined in filtered aliquots.

[2] Omitted from average.

is small and seems to confirm Engel's observation in this respect. This period was therefore chosen for the remainder of the work.

The second method consisted in placing 50 gm. of soil upon a folded filter and adding 8 c.c. (136 mg. NH_3) of a normal ammonium sulfate solution. After bringing the soil to optimum moisture conditions it was allowed to stand for 4 hours and leached with distilled water. In order to determine how much water was necessary to use in getting the fixation, a preliminary test was run. Consecutive 200-c.c. portions of the filtrate were collected and examined for ammonia. The results are shown in Table II.

Practically all of the ammonia that was recovered in the leachings was washed out in the first 200 c.c.; undoubtedly the addition indefinitely of more water to the soil would continue to bring out small amounts of ammonia. For the present purposes, however, 600 c.c. appeared to be sufficient. Soil No. II showed a rather interesting behaviour in that it continued to give out the most ammonia after the first 200 c.c. had passed

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through the soil. Throughout the whole work this soil shows exceptional characteristics of which special note should be taken. The ammonia fixed in the soil was determined by distillation with magnesium oxide. The amounts obtained are shown in the table, together with the total recovery.

TABLE II

RECOVERY OF AMMONIA ADDED TO SOIL[1]

Soil No.	1st 200 c.c. Ammonia		2nd 200 c.c. Ammonia		3rd 200 c.c. Ammonia		4th 200 c.c. Ammonia		5th 200 cc. Ammonia		% NH_3 in 1000 c.c. Av.	In Soil Ammonia		Total % Recovery
	Mg.	Av. Mg.	Mg.	Av. Mg.	Mg.	Av. Mg.	Mg.	Av. Mg.	Mg.	Av. Mg.		Av. Mg.	Mg.	
I	125.0	.....	1.4	....	1.0	....	0.8	....	0.4	....	....	1.0	....	....
	126.0	125.5	1.2	1.3	1.7	1.4	1.4	1.1	0.4	0.4	94.7	1.6	1.3	95.4
II	88.5	.....	4.1	....	2.5	....	2.4	....	2.1	....	....	23.5	....	....
	93.5	91.0	4.6	4.4	2.4	2.5	2.5	2.5	2.1	2.1	75.4	23.5	23.5	92.7
III	116.0	.....	3.6	....	1.7	....	1.2	....	1.0	....	....	8.8	....	....
	112.0	114.0	2.5	3.1	1.2	1.5	1.0	1.1	0.7	0.9	88.8	9.8	9.3	95.4
IV	116.0	.....	2.5	....	2.4	....	1.2	....	0.8	....	....	7.5	....	....
	114.0	115.0	2.7	2.6	1.2	1.8	0.8	1.0	0.8	0.8	89.2	7.4	7.5	94.6
V	109.0	.....	2.4	....	1.0	....	1.0	....	0.7	....	....	13.0	....	....
	102.0	105.5	4.0	3.2	1.2	1.1	1.0	1.0	0.7	0.7	81.4	13.0	13.0	91.9
VI	119.0	.....	2.2	....	1.0	....	0.8	....	0.7	....	....	7.5	....	....
	117.0	118.0	2.2	2.2	0.8	0.9	0.8	0.8	0.5	0.6	90.2	7.1	7.3	95.6

[1] 50-gm. portions of soil, 8 c.c. N/1 NH_3 added, brought to optimum moisture, stood 1 hour and leached, 136 mg. NH_3 added.

Not all the ammonia added was recovered, the percentage varying with the soils, as would be expected. Whether the absorption could best be determined by distilling off the ammonia "fixed" in the soil or by getting that in the filtrate was the next point studied.

TABLE III

RECOVERY OF AMMONIA ADDED TO DIFFERENT SOILS[1]

Soil No.	I		II		III		IV		V		VI	
	NH_3 Found		NH_3 Found		NH_3 Found		NH_3 Found		NH_3 Found		NH_3 Found	
	Mg.	Av. Mg.	Mg.	Av. Mg.	Mg.	Av. Mg.	Mg.	Av. Mg.	Mg.	Av. Mg.	Mg.	Av. Mg.
	16.1	.....	16.3	.....	15.8	.....	14.2	.....	15.3	.....	15.3	.....
	16.1	16.1	16.5	16.4	15.7	15.75	13.9	14.05	14.9	15.1	15.4	15.35
% Recov'd	.....	95.0	.....	96.5	.....	92.80	.....	82.7	.....	88.8	.....	90.3

[1] 1 c.c. N/1 NH_3 added to 50 gm. soil, allowed to stand 4 hours and NH_3 distilled off; 17 mg. NH_3 added.

In Table III the recovery of ammonia added is tabulated. It is seen that the recovery varies widely with the soil, indicating that the determination of the ammonia in the filtrate would probably be the most satisfactory criterion of the absorption.

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Having settled the minor considerations, a comparison of the two methods was carried out, 50 gm. of soil and 34 mg. of NH_3 being used in each case. The ammonia after standing in contact with the soil for 4 hours was determined in a filtered aliquot of the solution from the flask in the first method. In the second, the ammonia was determined in 200 c.c. of the first 600 c.c. of the leachings. The absorption was calculated and recorded in Table IV.

TABLE IV

COMPARISON OF THE ABSORPTION OF AMMONIA BY SOIL IN FLASKS AND UPON FILTERS

Soil No.	Lab. No.	50 gm. 200 c.c. N/100 NH_3 in flasks Ammonia Absorbed		Lab. No.	50 gm. 2 c.c. N/1 NH_3 on filters Ammonia Absorbed	
		Mg.	Av. Mg.		Mg.	Av. Mg.
I	157	1.4	.....	169	4.4	.....
	158	1.4	1.40	170	2.4	3.40
II	159	14.6	.....	171	16.6	.....
	160	13.9	14.25	172	18.0	17.30
III	161	9.2	.....	173	11.8	.....
	162	9.4	9.30	174	11.8	11.80
IV	163	7.8	.....	175	9.5	.....
	164	8.2	8.00	176	9.8	9.65
V	165	10.9	.....	177	11.5	.....
	166	10.9	10.90	178	14.1	12.80
VI	167	6.6	.....	179	8.5	.....
	168	6.3	6.45	180	8.5	8.50

The individual results are for the most part much more uniform in the series in which the flasks were used. It is true that a somewhat higher absorption is indicated with the second method, but the difference does not seem to be great enough to be a decided point in its favor, and it is fully

TABLE V

ABSORPTION OF AMMONIA BY SOILS

Soil	Mg. NH_3 per 50 gm. Soil	Pounds $(NH_4)_2SO_4$ per Acre
I. Norfolk sand	1.4	266
II. Collington sandy loam	13.9	2645
III. Sassafras loam	9.0	1710
IV. Dutchess silt loam	8.0	1520
V. Penn loam	10.5	1990
VI. Alloway clay	6.8	1290

counter-balanced by the lack of uniformity in the cases of Soils I, II and V. With one exception, the remainder of the work was carried out according to the first method.

It is natural to expect certain wide absorption ranges among soils of widely differing characters. We would undoubtedly expect that, other things being equal, the heavier the soil the greater would be its absorptive capacity. That this is true to a certain extent has been observed, but

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just what is the absorptive power of a specific soil can be determined only by trial. In Table V the relative absorption of ammonia is recorded both per 50 gm. of soil and per acre 6 2/3 inches (2,000,000 pounds of soil). With the exception of Soil I, the absorption by all soils appears to be sufficiently great that it would not be a factor of practical importance. The indication is undoubtedly a true one that very little nitrogen, as ammonia, would be leached out of any of the rest of these soils.

But the problem has a broader bearing than this, inasmuch as high absorptive powers of soils usually indicate high fertility. Following in the same manner that of potash, the absorption of ammonia probably is more truly indicative of the fertility of a soil than the absorption of phosphoric acid, since the absorption of the latter is largely due to the formation of insoluble compounds with iron and alumina. Most soils, even relatively infertile ones, possess enough of these constituents to absorb considerable quantities of phosphoric acid.

TABLE VI

MECHANICAL ANALYSIS OF SOILS

	Soil	Organic Matter	Gravel 2-1 mm.	Coarse Sand 1-.05 mm.	Medium Sand .5-.25 mm.	Fine Sand .25-.1 mm.	Very Fine Sand .1-.05 mm.	Silt .05-.005 mm.	Clay .005 mm.
		%	%	%	%	%	%	%	%
I.	Norfolk sand[1]	0.10	4.32	26.90	29.80	33.84	1.86	2.40	0.74
II.	Collington sandy loam[1]..	1.07	1.34	8.64	27.84	35.62	15.24	7.22	3.86
III.	Sassafras loam[1]	1.45	2.26	8.28	6.30	9.94	10.08	53.38	8.80
IV.	Dutchess silt loam[2].....	....	2.90	4.10	3.10	10.00	17.30	45.40	17.00
V.	Penn loam[1]	2.39	1.48	3.22	2.78	5.86	13.12	61.42	11.46
VI.	Alloway clay[1]	3.46	1.18	3.52	3.80	5.42	5.84	53.80	25.30

[1] Soil survey of the Trenton area, New Jersey. U. S. Dept. Agr. Field Operations Bur. Soils, 1902, p. 163-186.

[2] The mechanical and chemical composition of the soils of the Sussex area, New Jersey. Geol. Surv. N. J. Bul. 10, 1913.

A wide range of absorption is noted in Table V, the least occurring in the lightest soil, the Norfolk sand, and the most, not in the heaviest soil, but in the next lightest, the Collington sandy loam. The soils may be arranged according to their absorptive capacity from the greatest to the least as follows: II, V, III, IV, VI, I. That the Collington (Soil II) should occupy first place seemed almost inexplicable.

It was thought that possibly the mechanical and chemical analyses[1] of the respective soil types might aid in explaining the relative positions of the soils. Table VI, giving the mechanical analyses, apparently offers no clue, for there does not seem to be a single correlation between the absorption noted and any of the factors given in the table. But by barring

[1] Analyses of the soils were not made, since they represented common types of which analyses were available.

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Soil II the general tendency appears to show greater absorption in the heavier soils, although it seems evident that the mechanical composition can have only a minor influence. In the earlier work, where a correlation between this and the absorptive capacity appeared to hold, it is believed that other more important and potent elements were absent.

That absorption may take place even in such inert material as pure quartz sand is shown in Table VII. The only necessary condition to this apparently physical phenomenon is a fine state of division.

No absorption of ammonia could be detected up to 30-mesh sand when only 0.3 mg. was fixed; even in the finest quartz flour the absorption was small. Since quartz sand is probably the most resistant mineral in soils, it is doubtful if it reaches so fine a state of division in sufficient quantities to affect their absorptive properties to any extent. But it is not impossible that some of the absorption may be traced to this element.

TABLE VII
ABSORPTION OF AMMONIA BY SAND

Lab. No.	Fineness	Ammonia Absorbed	
		Mg.	Av. Mg.
181	16 mesh	0	...
182	16 mesh	0	0
183	24 mesh	0	...
184	24 mesh	0	0
185	30 mesh	.3	...
186	30 mesh	.3	.3
187	80 mesh	1.0	...
188	80 mesh	1.0	1.0
189	I5 XX bolting	2.0	...
190	Cloth	2.0	2.0

It is, however, quite possible and even probable that of two soils having like chemical constitution, the heavier one would manifest greater absorptive power.

Correlations between absorption and iron, alumina, calcium, magnesium, and carbon dioxide contents have frequently been pointed out. In Table VIII we find that in order of iron and alumina content the soils arrange themselves from highest to lowest in the following manner: V, IV, III, VI, II, I. By eliminating Soil II and interchanging Nos. III and IV, which show very close values, we get them in the order of their absorptive capacities. Whether this correlation is accidental or not, there still seems to be some relationship.

But with reference to calcium we find the order, II, V, III, IV, VI, I, and here we have the exact order of their absorptive powers. The presence of CaO then seems to affect strongly this property of soils.

Taking magnesia next, we find the order IV, V, VI, III, II, I, in which there seems to be no relationship. With carbon dioxide we get the following arrangement: III, IV, V, II, VI, I. The order III, IV, VI, I

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correlates very nicely, but the position of II and V rather invalidates this as any indication. With reference to the alkalies the order is: V, IV, III, VI, II, I, to which no significance is attached. According to the percentage of organic matter the soils are placed: V, IV, III, VI, II, I, which is the same order as the iron and alumina content and follows the absorption in the same manner.

TABLE VIII

CHEMICAL ANALYSIS OF SOILS

	I Norfolk[1] Sand %	II Colling'n[1] Sandy Loam %	III Sassafras[1] Loam %	IV Dutchess[2] Silt Loam %	V Penn[1] Loam %	VI Alloway[3] Clay %
Insoluble Matter	93.67	93.56	86.84	80.07	76.99	88.28
Soluble Silica	.01	.04	.03	.062	.08	.09
K_2O	.02	.07	.19	.315	.78	.17
Na_2O	.05	.05	.12	.057	.29	.08
CaO	.04	[4].28	.20	.107	.23	.06
MgO	.05	.05	.17	.910	.84	.22
Mn_3O_4	.02	.01	.03	.065	.08	.05
Fe_2O_3	1.96	1.32	2.51	3.917	4.02	2.12
Al_2O_3	.63	1.07	3.88	5.707	7.18	3.72
P_2O_5	.05	.132	.158	.186	.116	.08
SO_3	.05	.02	.08	.060	.04	.06
CO_2	.01	.025	.05	.034	.03	.024
Volatile Matter	3.08	3.27	5.75	6.21	9.24	4.98

[1] Lipman, J. G., and Blair, A. W. Investigations relative to the use of nitrogenous plant foods, 1898-1912. N. J. Agr. Exp. Sta. Bul. 288, 1916.

[2] The mechanical and chemical composition of the soils of the Sussex Area, New Jersey. Geol. Surv. N. J. Bul. 10, 1913.

[3] Lipman, J. G., and Blair, A. W. Field experiments on the availability of nitrogenous fertilizers. N. J. Agr. Exp. Sta. Bul. 260, 1913.

[4] Owing to the fact that this soil had been limed, the calcium was determined and substituted for the value given.

By way of chemical composition, then, it perhaps may be said of the six soils used, that there is a close relationship between the lime content and the absorption of ammonia. There exist also more or less close parallels between the contents of magnesia, alkalies, iron and alumina, carbon dioxide, and volatile matter, but these are perhaps less vital than other factors in determining the soil's absorptive power.

As a further effort to learn if hygroscopic moisture, apparent specific gravity, water-holding capacity, or lime requirement bore any relation to the absorption of ammonia, these were determined and the results given in Table IX.

No direct relationship can be assigned to these factors except in a general way. For instance, the smallest amount of hygroscopic moisture is shown by Soil I, having the least absorption, and the largest amount, barring Soil II, is shown by Soil V, having the greatest absorption.

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Roughly the absorption is inversely proportional to the apparent specific gravity and proportional to the water-holding capacity with the one exception. All the soils, except No. II, shows a lime requirement. This fact at once seemed to afford a possible explanation of its peculiar behavior which resulted in its occupying first place with reference to its absorptive power.

TABLE IX

HYGROSCOPIC MOISTURE, APPARENT SPECIFIC GRAVITY, WATER-HOLDING CAPACITY AND LIME REQUIREMENT OF SOILS USED

	I	II	III	IV	V	VI
Soil No.	Norfolk Sand	Colling'n Sandy Loam	Sassafras Loam	Dutchess Silt Loam	Penn Loam	Alloway Clay
Hygroscopic Moisture, %...	.15	1.60	1.31	1.87	2.28	.88
Apparent Specific Gravity...	1.52	1.34	1.18	1.16	1.10	1.30
Water-Holding Capacity, %.	.34	.42	.49	.54	.55	.44
Lime Requirement—lbs. CaO per 2,000,000 lbs. soil....	550	Alkaline	1400	2200	1500	1200

If, then, the fact that the reaction of one soil was basic was responsible for its superior absorptive capacity, there seemed to be no reason why the addition of lime to the other soils should not decidedly raise their absorption.

TABLE X

EFFECTS OF CaO AND $CaCO_3$ UPON ABSORPTION OF AMMONIA

	No Treatment Ammonia Absorbed			0.1 gm. CaO to 50 gm. Soil Ammonia Absorbed				0.2 gm. CaO_3 to 50 gm. Soil Ammonia Absorbed			
Soil No.	Lab. No.	Mg.	Av. Mg.	Lab. No.	Mg.	Av. Mg.	Increase Av. Mg.	Lab. No.	Mg.	Av. Mg.	Increase Av. Mg.
I	227	1.4	.....	275	3.7	.....	.....	287	3.4	.....	.....
	228	1.4	1.4	276	4.4	4.05	2.65	288	3.7	3.55	2.15
II	229	13.9	.....	277	10.2	.....	.....	289	14.6	.....	.....
	230	13.9	13.9	278	10.2	10.20	—3.70	290	14.6	14.60	0.70
III	231	8.8	.....	279	11.9	.....	.....	291	9.9	.....	.....
	232	9.2	9.0	280	11.7	11.80	2.80	292	9.9	9.90	0.90
IV	233	8.2	.....	281	11.4	.....	.....	293	9.3	.....	.....
	234	7.8	8.0	282	11.5	11.45	3.45	294	8.7	9.00	1.00
V	235	10.5	.....	283	12.9	.....	.....	295	11.2	.....	.....
	236	10.5	10.5	284	11.9	12.40	2.35	296	.....	11.2	0.70
VI	237	6.5	.....	285	9.5	.....	.....	297	7.5	.....	.....
	238	7.1	6.8	286	9.9	9.70	2.90	298	7.5	7.5	0.70

Accordingly 0.1 gm. of CaO was added to one set of soils and 0.2 gm. $CaCO_3$ was added to another set, and the absorption determined as previously indicated. In Table X the results are recorded and figure 1 shows graphically the effect of the former. The addition of calcium carbonate, while serving to increase slightly the capacity of all soils to absorb am-

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monia, was not marked in its action and would lead us to believe that it does not function actually in the absorption process.

In the case of the calcium oxide, however, we can note a decided increase in the soils' absorptive capacity for ammonia in every case but one, that of No. II. Why this soil should behave in this manner was not at first clear. It seemed reasonable to suppose that even though there was some free CaO in the soil before, the addition of more would strengthen its absorptive power. But since this was not the case, the only evident explanation seemed that there was possibly an over-abundance of the element in the soil already. This might tend to indicate that there is a point with every soil where the addition of CaO would effect a lowering of its absorptive capacity.

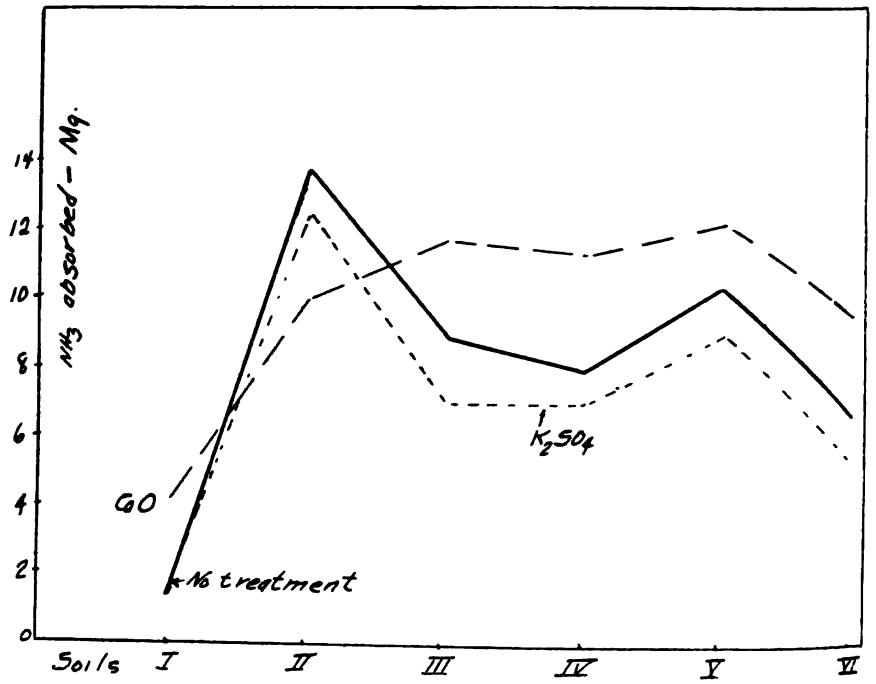

Fig. 1.—Diagram showing the effect of CaO and K_2SO_4 upon the absorption of ammonia.

Quantities of CaO ranging from 0.1 gm. up to 0.5 gm. were added to each soil and the absorption again noted. The results are shown numerically and graphically in Table XI and figure 2.

Exactly what we anticipated took place. There is shown at first in every case but that of Soil II, an increase in the absorptive capacity followed by a subsequent steady decrease. It is interesting to see that the absorption of Soil II decreases in almost a straight line until with 0.3 gm. CaO it absorbs only as much as Soil I, that is, the least amount; that this

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reaches a constant with both soils below which it does not go is indicative, perhaps, that with a certain lime-content all the soils may finally reach a similar constant. The presence of lime, then, in sufficient quantities[1] almost paralyzes the absorption mechanism. At any rate it shows that even it alone can be a limiting factor before which all others must give way. But it is doubtless true that in most soils little if any calcium would be present as CaO and the absorption in these soils must be due principally to other causes. This fact serves to strengthen the writer's view that the process is a very complicated one where one factor, in the absence of others, may be responsible for the bulk of the absorption.

TABLE XI

EFFECT OF INCREASING AMOUNTS OF CaO UPON THE ABSORPTION OF AMMONIA BY SOILS

Gm. CaO per 50 gm. Soil	Soil I			Soil II			Soil III		
	Lab. No.	Ammonia Absorbed		Lab. No.	Ammonia Absorbed		Lab. No.	Ammonia Absorbed	
		Mg.	Av. Mg.		Mg.	Av. Mg.		Mg.	Av. Mg.
None	299	1.4	..... Inc. due to	311	12.9	..... Inc. due to	323	8.5	..... Inc. due to
None	300	1.4	1.4 CaO	312	12.9	12.9 CaO	324	8.5	8.5 CaO
0.1	301	2.4	....	313	9.5	....	325	10.9	....
0.1	302	2.0	2.2 0.8	314	9.5	9.5 —3.4	326	10.7	10.8 2.3
0.2	303	1.7	....	315	5.4	....	327	11.0	....
0.2	304	1.7	1.7 0.3	316	4.8	5.1 —7.8	328	11.0	11.0 2.5
0.3	305	1.7	....	317	1.7	....	329	9.9	....
0.3	306	1.7	1.7 0.3	318	1.7	1.7 -11.2	330	9.9	9.9 1.4
0.4	307	1.7	....	319	1.7	....	331	8.5	....
0.4	308	1.7	1.7 0.3	320	1.7	1.7 -11.2	332	8.5	8.5 0.0
0.5	309	1.7	....	321	1.7	....	333	6.6	....
0.5	310	1.7	1.7 0.3	322	1.7	1.7 -11.2	334	6.8	6.7 —1.8
	Soil IV			Soil V			Soil VI		
None	335	7.8	....	347	9.5	....	359	6.8	....
None	336	7.2	7.5	348	9.7	9.6	360	6.6	6.7 .....
0.1	337	10.6	....	349	11.8	....	361	9.5	....
0.1	338	11.2	10.4 2.9	350	12.2	12.0 2.4	362	9.5	9.5 2.8
0.2	339	11.8	....	351	13.5	....	363	8.5	....
0.2	340	11.2	11.5 4.0	352	13.3	13.4 3.8	364	8.5	8.5 1.8
0.3	341	11.8	....	353	12.6	....	365	6.8	....
0.3	342	11.0	11.4 3.9	354	12.8	12.7 3.1	366	6.6	6.7 .0
0.4	343	10.5	....	355	11.8	....	367	4.8	....
0.4	344	10.9	10.7 3.2	356	11.6	11.7 2.1	368	5.2	5.0 —1.7
0.5	345	9.6	....	357	11.1	....	369	4.1	....
0.5	346	8.8	9.2 1.7	358	10.9	11.0 1.4	370	4.1	4.1 —2.6

Flocculation and deflocculation in soils form a basis for the discussion of the problem from another standpoint. It is well known that deflocculation follows the excess of soluble bases in the soil which tend toward favoring the presence of large amounts of colloidal material; on the other hand, the presence of acids usually indicates a minimum of colloids,

[1] With the larger amounts of CaO used some volatilization of ammonia took place, but in no case did it exceed 2 mg.

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owing to hydrolysis. The supporters of the colloidal theory of absorption have laid considerable stress upon this point. The matter of reaction is therefore a point to be considered.

Table XII with figure 3 shows the effect upon absorption of a small amount of acid and alkali, 2 c.c. of normal HCl being added to one series of soils and 2 c.c. normal NaOH to the other series.

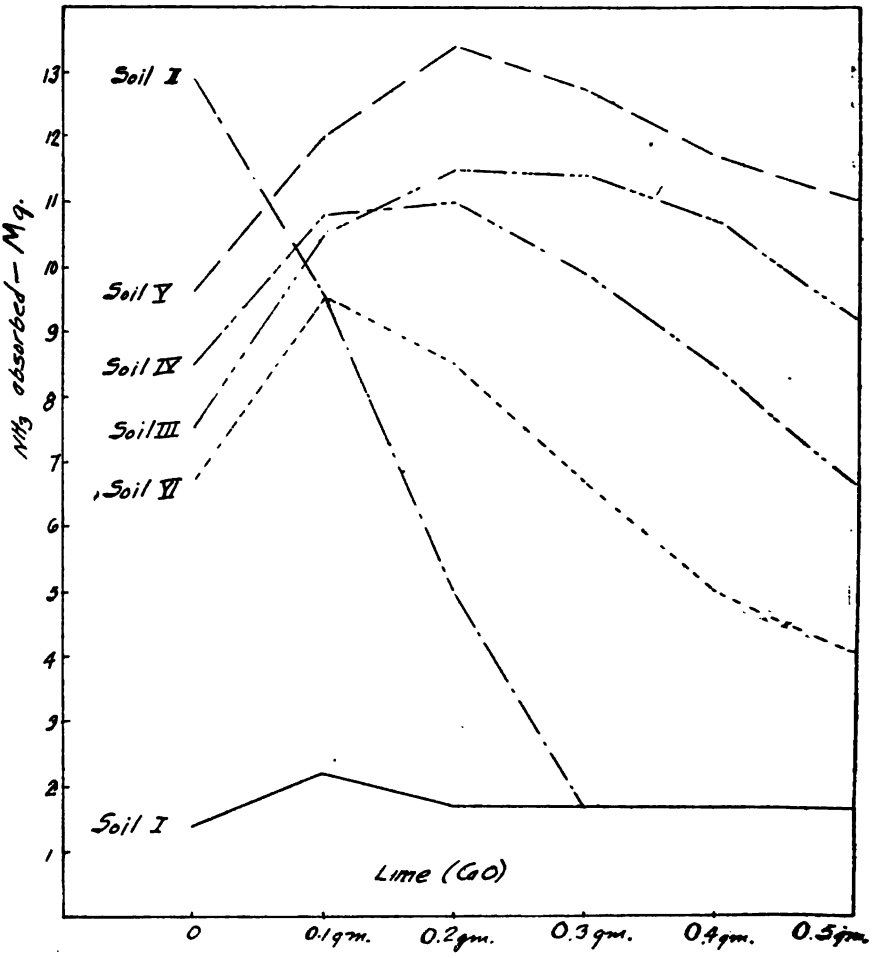

Fig. 2.—Diagram showing the effect of increased amounts of CaO upon the absorption of ammonia.

In every case we get a fairly large increase due to the presence of NaOH. This is undoubtedly because of the deflocculating effect of the base in the probable formation of colloids such as $Fe(OH)_3$, $Al(OH)_3$, various silicates and numerous others. That this increase is largely due to the formation of colloidal iron and alumina is indicated by the fact that the increase is marked in Soil I, where there is very little clay and

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TABLE XII

EFFECT OF SODIUM HYDROXIDE AND HYDROCHLORIC ACID ON ABSORPTION OF AMMONIA

Soil No.	No Treatment Ammonia Absorbed			2 c.c. Normal NaOH Ammonia Absorbed				2 c.c. Normal HCl Ammonia Absorbed			
	Lab. No.	Mg.	Av. Mg.	Lab. No.	Mg.	Av. Mg.	Increase Av. Mg.	Lab. No.	Mg.	Av. Mg.	Increase Av. Mg.
I	227	1.4	.....	251	6.1	.....	.....	263	0.2	.....	.....
	228	1.4	1.4	252	6.1	6.10	4.70	264	0.3	0.25	—1.15
II	229	13.9	.....	253	11.6	.....	.....	265	9.5	.....	.....
	230	13.9	13.9	254	12.2	11.90	2.00	266	9.9	9.70	—4.20
III	231	8.8	.....	255	15.3	.....	.....	267	4.1	.....	.....
	232	9.2	9.0	256	15.0	15.15	6.15	268	4.1	4.10	—4.90
IV	233	8.2	.....	257	15.6	.....	.....	269	2.4	.....	.....
	234	7.8	8.0	258	15.3	15.45	7.45	270	3.1	2.75	—5.25
V	235	10.5	.....	259	16.3	.....	.....	271	5.5	.....	.....
	236	10.5	10.5	260	17.3	16.80	6.30	272	4.4	4.95	—5.55
VI	237	6.5	.....	261	11.2	.....	.....	273	1.7	.....	.....
	238	7.1	6.8	262	8.8	10.0	3.20	274	2.4	2.05	—4.75

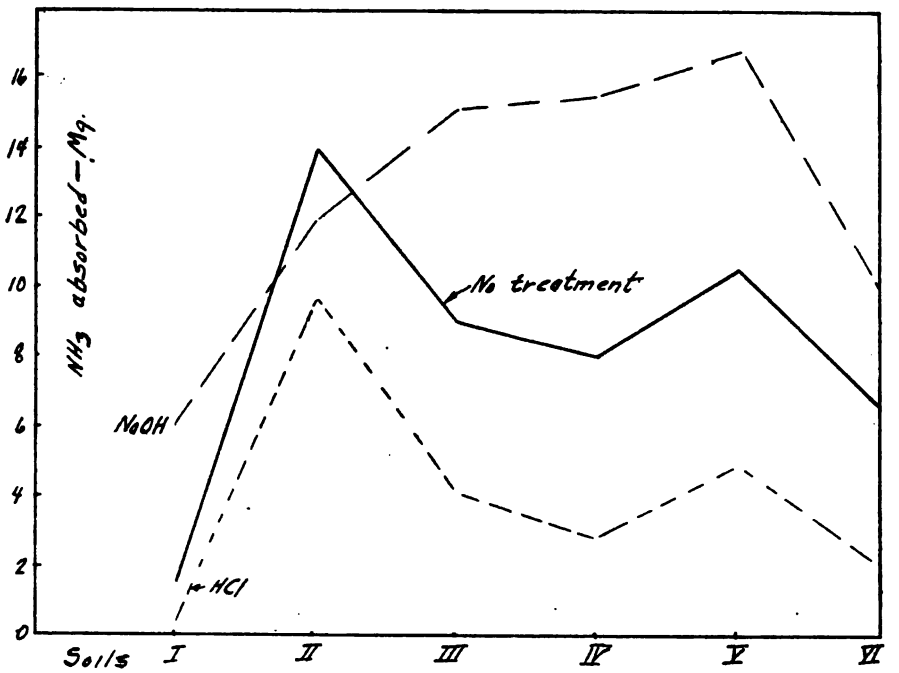

Fig. 3.—Diagram showing the effect of reaction of solution upon the absorption of ammonia.

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the soil consists almost entirely of silica, iron and alumina. The lowest increase, it is noted, is for the soil having the lowest lime requirement (basic), and the largest increase for the soil having the highest lime requirement.

Just as we obtained an increase in the absorptive capacity by the introduction of NaOH, in the same way we get a decrease in every case by the use of HCl. This may be due to the hydrolysis of the soil colloids as well as to the neutralization of any bases that might have been present.

TABLE XIII

EFFECT OF WASHING OUT WATER-SOLUBLE AND ACID-SOLUBLE[1] MATTER UPON ABSORPTION OF AMMONIA BY SOILS UPON FILTERS

Soil No.	Ammonia Absorbed No Treatment			Ammonia Absorbed Leached with 600 c.c. H_2O				Ammonia Absorbed 25 c.c. N/1 HCl + 500 c.c. H_2O			
	Lab. No.	Mg.	Av. Mg.	Lab. No.	Mg.	Av. Mg.	Increase Av. Mg.	Lab. No.	Mg.	Av. Mg.	Increase Av. Mg.
I	191	2.9	.....	203	3.4	.....	.....	215	1.6	.....	.....
	192	4.5	3.70	204	3.7	3.55	—0.15	216	2.6	2.10	—1.60
II	193	15.9	.....	205	19.7	.....	.....	217	11.8	.....	.....
	194	17.2	16.55	206	21.7	20.70	4.15	218	11.6	11.70	—4.85
III	195	10.8	.....	207	.....	.....	.....	219	2.9	.....	.....
	196	10.8	10.80	208	10.5	10.50	—0.30	220	4.4	3.65	—5.15
IV	197	13.1	.....	209	12.1	.....	.....	221	7.0	.....	.....
	198	9.9	11.50	210	10.5	11.30	—0.20	222	9.2	8.10	—3.40
V	199	14.1	.....	211	16.1	.....	.....	223	8.0	.....	.....
	200	13.6	13.85	212	16.6	16.35	2.50	224	6.0	7.00	—6.85
VI	201	8.0	.....	213	9.5	.....	.....	225	[2]0.1	.....	.....
	202	9.0	8.50	214	9.5	9.50	1.00	226	3.4	3.40	—5.10

[1] 25 c.c. normal HCl added to 50 gm. soil in flasks, digested on a water bath for 1 hour, and leached with 500 c.c. distilled water.

[2] Omitted from average.

It was thought that by washing out the water-soluble and acid-soluble matter from the soils, the causes of absorption might be further limited. In Table XIII the results of treating with 600 c.c. of distilled water and also by digestion with 25 c.c. normal HCl for an hour, filtering and washing, are presented. It is realized that very little of the soluble salts would be leached out in so small a quantity of water, yet it seemed to offer another point of attack.

In this experiment the leaching method was used to determine the absorption. It will be noted that in three cases the absorption is greater after leaching than before; while there is a slight decrease in the other three instances, this may well be within the limits of error. At any rate the absorption does not appear to be impaired, but rather enhanced. It is perhaps allowable to say that absorption is not increased by the presence of easily soluble salts.

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However, the loss of acid-soluble salts including the hydrolyzable colloids appreciably decreased the absorptive powers of the soils. It is to be noted by comparison with Table XII that the decrease in absorption is much more marked, a condition due probably as much to the greater amount of acid used as to the removal of the salts from the soil.

The digestion with acid was repeated, 50 c.c. of concentrated HCl and 50 gm. of soil being used. After allowing the extraction to take place on the water bath for 2 hours the acid was decanted off and the soil was washed upon a filter with 500 c.c. of water. The absorption was determined in the flasks as usual. The results are given in Table XIV.

TABLE XIV

EFFECT OF REMOVAL OF ACID-SOLUBLE MATTER UPON ABSORPTION OF AMMONIA BY SOILS

Soil No.	No Treatment			Treatment[1]			Decrease due to Treatment
	Lab. No.	Ammonia Absorbed		Lab. No.	Ammonia Absorbed		
		Mg.	Av. Mg.		Mg.	Av. Mg.	Av. Mg.
I	227	1.4	....	239	0.7	....	....
	228	1.4	1.4	240	0.7	0.70	0.70
II	229	13.9	....	241	9.5	....	....
	230	13.9	13.9	242	9.2	9.35	4.65
III	231	8.8	....	243	2.4	....	....
	232	9.2	9.0	244	2.7	2.55	6.45
IV	233	8.2	....	245	2.0	....	....
	234	7.8	8.0	246	2.0	2.00	6.00
V	235	10.5	....	247	1.7	....	....
	236	10.5	10.5	248	2.4	2.05	8.45
VI	237	6.5	....	249	1.7	....	....
	238	7.1	6.8	250	1.7	1.70	5.10

[1] 50 gm. digested upon water bath for 2 hours with 50 c.c. concentrated HCl, filtered and washed with 500 c.c. water.

It is rather surprising to observe that even less decrease was effected in the case of Soil I by this drastic treatment than by the use of 2 c.c. of normal acid shown in Table XII. For the most part, however, the treatment shows greater effect than the previous one, indicating that a gradual solution of the soil constituents lowered the absorptive capacity.

As a means of changing soil structure, particularly that due to colloids, the agency of heat can be advantageously employed. Various other changes take place as well in the drying of soils, but it was thought that this procedure would give some indications worth while. Samples of all six soils were heated in a hot-air oven at 117° C. for 5 hours, after which they were treated with ammonia as usual. The effect of this treatment as recorded in Table XV shows a decrease in every case, the extent varying almost exactly in proportion to the water-holding capacities of the soils. The water-holding capacity is perhaps to some extent a function of the colloids in a soil and it is rather to be expected that the loss of water at high temperatures would destroy or incapacitate them in this

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respect. The decreases shown appear to be due to this cause. The low decrease in the case of Soil II confirms the previous view that most of the absorption was due to CaO.

If the use of heat is still further employed to the extent of burning out the organic matter of the soils, other factors are surely eliminated. Portions of each soil were placed in iron crucibles and burned at redness for 30 minutes. Table XV gives the results obtained.

TABLE XV

EFFECT OF DRYING AND BURNING OF SOILS UPON THEIR ABSORPTION OF AMMONIA

Soil No.	No Treatment Ammonia Absorbed			Dried At 117° C. for 5 Hours Ammonia Absorbed				Organic Matter Burned Out at Red Heat Ammonia Absorbed			
	Lab. No.	Mg.	Av. Mg.	Lab. No.	Mg.	Av. Mg.	Increase Av. Mg.	Lab. No.	Mg.	Av. Mg.	Increase Av. Mg.
I	227	1.4	.....	383	0.9	.....	.....	395	0.3	.....	.....
	228	1.4	1.4	384	0.9	0.90	—0.50	396	0.7	0.50	—0.90
II	229	13.9	.....	385	7.1	.....	.....	397	11.6	.....	.....
	230	13.9	13.9	386	6.8	6.95	—6.95	398	12.6	12.10	—1.80
III	231	8.8	.....	387	4.8	.....	.....	399	6.1	.....	.....
	232	9.2	9.0	388	3.4	4.10	—4.90	400	5.4	5.75	—3.25
IV	233	8.2	.....	389	3.6	.....	.....	401	1.7	.....	.....
	234	7.8	8.0	390	3.2	3.40	—4.60	402	1.7	1.70	—6.30
V	235	10.5	.....	391	2.7	.....	.....	403	2.7	.....	.....
	236	10.5	10.5	392	2.7	2.70	—7.80	404	2.0	2.35	—8.15
VI	237	6.5	.....	393	1.7	.....	.....	405	2.5	.....	.....
	238	7.1	6.8	394	1.7	1.70	—5.10	406	2.5	2.50	—4.30

Practically every soil shows a large decrease due to this treatment except Soil I, where on account of its nature not much change would be expected. In the case of Soil II it will be remembered that it already had a large percentage of free CaO and the burning undoubtedly calcined more, so that a decrease in absorption took place. The destruction of the soil structure is perhaps responsible for the lowering of the absorption in the other cases. An interesting point to note is that in the cases of Soils I, IV and V the burning produced a smaller effect than merely drying. Inasmuch as the soil had first to pass through the drying stage before burning it must have been lowered as much as indicated by the former process. It was then necessary that some compensating action take place to restore the condition that effected the results obtained by burning. This might possibly be explained by assuming that there was some basicity produced by the burning. The loss of organic matter by burning appears to be of much less consequence than the loss of water.

This is confirmed in Table XVI, where the effects of the oxidation of the soil organic matter by nitric acid are shown. Fifty-gram portions of

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the soils were digested for 2½ hours over a water bath with 100 c.c. of concentrated HNO_3. After filtering and washing with 1000 c.c. of distilled water the absorption was determined.

TABLE XVI

EFFECT OF OXIDATION OF ORGANIC MATTER BY NITRIC ACID UPON THE ABSORPTION OF AMMONIA BY SOILS

Soil No.	No Treatment			100 cc. Concentrated HNO_3			Decrease due to Treatment
	Lab. No.	Ammonia Absorbed		Lab. No.	Ammonia Absorbed		
		Mg.	Av. Mg.		Mg.	Av. Mg.	Av. Mg.
I	227	1.4	....	371	1.0	....	....
	228	1.4	1.4	372	1.0	1.00	0.40
II*	229	13.9	....	373	13.3	....	....
	230	13.9	13.9	374	13.3	13.30	0.60
III	231	8.8	....	375	6.1	....	....
	232	9.2	9.0	376	6.1	6.10	2.90
IV	233	8.2	....	377	7.5	....	....
	234	7.8	8.0	378	7.1	7.30	0.70
V	235	10.5	....	379	7.1	....	....
	236	10.5	10.5	380	7.5	7.30	3.20
VI	237	6.5	....	381	4.4	....	....
	238	7.1	6.8	382	5.5	4.95	1.85

It is surprising to observe that there is so little effect from this treatment; the organic matter was not only destroyed but the acid-soluble salts were removed as well. Not as much change is noted in this case as with

TABLE XVII

EFFECT OF ORGANIC MATTER (COTTONSEED MEAL) UPON THE ABSORPTION OF AMMONIA BY SOILS

Soil No.	No Treatment			1 gm. Cottonseed Meal added to 50 gm. Soil			Decrease due to Treatment
	Lab. No.	Ammonia Absorbed		Lab. No.	Ammonia Absorbed		
		Mg.	Av. Mg.		Mg.	Av. Mg.	Av. Mg.
I	227	1.4	....	407	3.1	....	....
	228	1.4	1.4	408	3.1	3.10	1.70
II	229	13.9	....	409	13.9	....	....
	230	13.9	13.9	410	14.3	14.10	0.20
III	231	8.8	....	411	9.5	....	....
	232	9.2	9.0	412	9.2	9.35	0.35
IV	233	8.2	....	413	8.5	....	....
	234	7.8	8.0	414	8.5	8.50	0.50
V	235	10.5	....	415	10.7	....	....
	236	10.5	10.5	416	10.7	10.70	0.20
VI	237	6.5	....	417	7.5	....	....
	238	7.1	6.8	418	7.1	7.30	0.50

the treatment with the relatively weak (normal) HCl as reported in Table XIII. The only explanation at present seems to be that perhaps the use of 1000 c.c. of wash water more nearly washed out the acid than

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the 500 c.c. used in the other instance. But even though the decrease is small the loss of the organic matter has a marked effect.

Increasing the organic matter by the use of cottonseed meal did not have a very great effect upon the absorption. This experiment was carried out by adding 1 gm. of the material to 50 gm. of soil, thoroughly mixing and treating with ammonia.

Although there was an increase in every case it was very small except with Soil I, which was lowest in organic matter. These increases are in all probability due principally to physical absorption.

TABLE XVIII

EFFECT OF CHLORIDE AND SULPHATE OF POTASSIUM UPON THE ABSORPTION OF AMMONIA BY SOILS

Soil No.	No Treatment Ammonia Absorbed			0.1 gm. K_2SO_4 Ammonia Absorbed				0.086 gm. KCl Ammonia Absorbed			
	Lab. No.	Mg.	Av. Mg.	Lab. No.	Mg.	Av. Mg.	Increase Av. Mg.	Lab. No.	Mg.	Av. Mg.	Increase Av. Mg.
I	227	1.4	.....	431	1.0	.....	.....	443	1.7	.....	.....
	228	1.4	1.4	432	1.7	1.35	—0.05	444	1.7	1.70	0.30
II	229	13.9	.....	433	12.6	.....	.....	445	11.9	.....	.....
	230	13.9	13.9	434	12.6	12.60	—1.30	446	11.2	11.55	—2.35
III	231	8.8	.....	435	7.5	.....	.....	447	6.8	.....	.....
	232	9.2	9.0	436	6.7	7.10	—1.90	448	7.1	6.95	—2.05
IV	233	8.2	.....	437	7.5	.....	.....	449	6.5	.....	.....
	234	7.8	8.0	438	6.8	7.15	—0.85	450	6.5	6.50	—1.50
V	235	10.5	.....	439	9.2	.....	.....	451	8.2	.....	.....
	236	10.5	10.5	440	9.2	9.20	—1.30	452	8.2	8.20	—2.30
VI	237	6.5	.....	441	5.6	.....	.....	453	5.1	.....	.....
	238	7.1	6.8	442	5.3	5.45	—1.35	454	5.1	5.10	—1.70

From the practical standpoint the methods of controlling the absorptive properties of soils are rather limited. The addition of lime, alteration of physical condition, application of organic matter and fertilizer salts constitute nearly all of the means available for the purpose. To determine the effect of the addition of potash and phosphoric acid upon the absorption of ammonia, experiments were carried out as follows: four separate sets (50-gm. portions) of soils were treated respectively with 0.1 gm. K_2SO_4; 0.086 gm. KCl (equivalent K_2O); 0.2 gm. acid phosphate; and 0.1 gm. K_2SO_4 and 0.2 gm. acid phosphate together. The results are given in Tables XVIII and XIX.

With only one exception, and that almost within experimental error, the use of potash[1] in either form caused a decrease in the absorption of ammonia. The decrease was, however, more marked in the case of the muriate. This is perhaps to be expected, inasmuch as this salt is more

[1] See figure 1.

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strongly absorbed by the soil than the sulfate. Both of these observations confirm similar ones made upon other soils.

Table XIX shows that in practically every case the use of acid phosphate tends to decrease slightly the soil's absorptive power for ammonia. However, a combination of acid phosphate and K_2SO_4 caused a considerable decrease in the absorption. This decrease was greater than the combined effect of the two substances acting alone.

The fact that potassium can replace ammonium and that the acid phosphate is acidic in character, are probably the causes of the lowering of the absorptive power.

TABLE XIX

EFFECT OF ACID PHOSPHATE ALONE AND WITH POTASH UPON THE ABSORPTION OF AMMONIA BY SOILS

Soil No.	No Treatment Ammonia Absorbed			0.2 gm. Acid Phosphate Ammonia Absorbed				0.1 gm. K_2SO_4 + 0.2 gm. A. P. Ammonia Absorbed			
	Lab. No.	Mg.	Av. Mg.	Lab. No.	Mg.	Av. Mg.	Increase Av. Mg.	Lab. No.	Mg.	Av. Mg.	Increase Av. Mg.
I	227	1.4	.....	455	1.7	.....	.....	467	0.7	.....	.....
	228	1.4	1.4	456	1.7	1.70	0.30	468	1.0	0.85	—0.55
II	229	13.9	.....	457	13.9	.....	.....	469	10.2	.....	.....
	230	13.9	13.9	458	13.6	13.75	—0.15	470	10.2	10.20	—3.70
III	231	8.8	.....	459	8.8	.....	.....	471	5.8	.....	.....
	232	9.2	9.0	460	9.0	8.90	—0.10	472	5.8	5.80	—3.20
IV	233	8.2	.....	461	7.5	.....	.....	473	4.8	.....	.....
	234	7.8	8.0	462	7.5	7.50	—0.50	474	4.8	4.80	—3.20
V	235	10.5	.....	463	9.9	.....	.....	475	6.6	.....	.....
	236	10.5	10.5	464	9.9	9.90	—0.60	476	7.1	6.85	—3.65
VI	237	6.5	.....	465	5.8	.....	.....	477	3.1	.....	.....
	238	7.1	6.8	466	5.8	5.80	—1.00	478	3.6	3.35	—3.45

Briefly reviewing the work in Part I, we find ourselves still further impressed with the complexity of the absorption phenomenon. In a simple way relationships were pointed out which indicated that the lime (CaO) content could be a strong factor in the absorption; that the contents of iron, alumina, magnesia, carbon dioxide, and organic matter, as well as mechanical composition, were undoubtedly correlated to some extent. That a large percentage of the absorption may be traced to the colloids and the finer soil particles seems clear. The soil organic matter, soluble matter and reaction are elements to be considered. The presence of KCl, K_2SO_4, or acid phosphate, decreases the soil's capacity for ammonia.

The phenomenon of absorption appears to be a complex of numerous factors of physical, physical-chemical, chemical, and perhaps biological nature, all of which react in one direction or another, depending upon the conditions of their balance.

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Part II

Factors Affecting the Vertical Distribution of Ammonia Applied to Soils

The vertical distribution of applied ammonia must bear some relation to its effectiveness. That this distribution is in a certain measure a function of the fixing or absorptive power of a soil is very probable. It appears, then, that by combining the study both of absorption and distribution of ammonia some more or less valuable information might be obtained.

Huston's statement that the absorption of a soil to a depth of 9 inches was more than 32 times as great as it would be called upon to exert in fixing a dressing of 500 pounds of ammonium sulfate would indicate that even the rather large application of 500 pounds could be fixed in a little more than a quarter of an inch of soil. Of course this would assume a perfectly even lateral distribution which could never be obtained. But even if the distribution were not made carefully the indication is that we might expect all of it to be fixed within the first inch. It will be remembered that of the soils used in this work the one with the highest fixing power was able to fix only about 2700 pounds of ammonium sulfate, and the one with the lowest, scarcely a tenth as much per acre 6 2/3 inches. Evidently the soils with which Huston worked had absorptive capacities far in excess of those possessed by the soils of New Jersey, even though many of these soils are exceptionally fertile. The problem of the distribution of applied ammonia is therefore a more practical one for New Jersey conditions.

Crawley and Duncan (6) have shown that in certain Hawaiian soils nearly one-half of the applied ammonia was fixed in the first two inches, nearly four-fifths in the first three inches, and nearly all in the first four inches.

But broad statements regarding the depth of fixation of ammonia in soils are undoubtedly misleading and of a little value; the individual conditions will make the problem a different one in every case. The work of which the following is a report does not warrant generalizations, but merely shows the range of results obtained and the effect of a few practical factors which can easily be controlled.

The work was carried out upon soils contained in paraffined paper tubes, having an inside diameter of 1¾ inches and a length of 8 inches. The tubes were closed at one end with paraffined corks and the soil column was approximately 6 2/3 inches in height. The weights of the respective soils were as follows:

Soil No. I	Soil No. II	Soil No. III	Soil No. IV	Soil No. V	Soil No. VI
Norfolk Sand	Collington Sandy Loam	Sassafras Loam	Dutchess Silt Loam	Penn Loam	Alloway Clay
380 gm.	335 gm.	295 gm.	290 gm.	275 gm.	325 gm.

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The ammonia was added to the surface of the soils in the tubes in 5 c.c. of a N/2 solution of ammonium sulfate (42.5 mg. NH_3), and water added to bring them to the desired moisture content.

In order to get the ammonia fixed or present at different depths, the tube was placed in an improvised miter box and cut into sections. The soil sections, as well as the cardboard rings, were then placed in copper distillation flasks and the ammonia distilled off by the use of magnesium oxide. All determinations were made in duplicate from which the corresponding blanks were subtracted.

That the vertical distribution would change somewhat with the time allowed for standing seemed quite probable and it therefore appeared necessary to run a preliminary test to determine a satisfactory period. For this test Soil IV, the Dutchess silt loam, was chosen because it represented a fairly heavy soil in which it was thought that movement of salts would be relatively slow. To three pairs of tubes containing this soil, ammonia and water were added. One pair was sectioned, and the ammonia determined on each of three consecutive days. The results recorded in Table XX show that the time element beyond the first day is

TABLE XX

EFFECT OF TIME UPON THE VERTICAL DISTRIBUTION OF AMMONIA[1]

No. Soil	Inches Depth	1 Day		2 Days		3 Days	
		Lab. No.	Ammonia Mg.	Lab. No.	Ammonia Mg.	Lab.	Ammonia Mg.
IV	0-1	483	13.9	489	12.9	495	13.0
	1-2	484	8.8	490	9.6	496	9.3
	2-3	485	8.0	491	8.0	497	7.5
	3-4	486	8.5	492	7.2	498	8.0
	4-5	487	2.4	493	3.1	499	3.7
	5-6 2/3	488	1.5	494	1.8	500	1.9

[1] Blanks not deducted.

not a very potent element in affecting the distribution of ammonia. However, the choice of the second day as the best length of time seemed warranted inasmuch as there was less change between the second and third days than between the first and second.

It was at first planned to leach the soils in the tubes and then note the position of the fixed ammonia, but after finding that the time necessary to do so was so variable with the soil, the idea was given up. To approximate, after a manner, the leaching effect of rain, it was decided to add the ammonia as before and then bring the soil to the desired moisture content by pouring the water in on top of the ammonia. The quantity of water to use was next studied. Three soils, Nos. I, III and V were used, representing the lightest, medium and heaviest. In one set of tubes the moisture content was brought to 50 per cent, in the next to 75 per cent,

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and in the third to 100 per cent of the water-holding capacity. After standing for two days the tubes were sectioned and the ammonia determined as before. In Table XXI we see that the increased moisture has the effect that we might expect. By glancing at figure 4 we can see perhaps a little more clearly that the lower moisture content favors a larger amount of ammonia in the surface layers. A moisture content of 50 per cent of the water-holding capacity was chosen for the subsequent work.

The wide variation in the vertical distribution of ammonia as shown in Table XXII is most interesting. Starting with Soil I, the lightest one,

TABLE XXI

EFFECT OF DIFFERENT MOISTURE CONTENTS OF SOILS UPON THE VERTICAL DISTRIBUTION OF AMMONIA[1] AT 2 DAYS

Soil No.	Depth Inches	50% Water-Holding Capacity		75% Water-Holding Capacity		100% Water-Holding Capacity	
		Lab. No.	Ammonia Mg.	Lab. No.	Ammonia Mg.	Lab. No.	Ammonia Mg.
I	0-1	501	2.0	516	1.9	531	1.0
	1-2	502	1.9	517	1.4	532	1.4
	2-3	503	4.4	518	1.7	533	1.5
	3-4	504	10.9	519	3.2	534	4.4
	4-6 2/3	505	25.2	520	31.4	535	32.8
III	0-1	506	15.1	521	12.9	536	10.7
	1-2	507	10.9	522	10.9	537	8.5
	2-3	508	8.8	523	7.0	538	7.1
	3-4	509	5.8	524	5.6	439	6.6
	4-6 2/3	510	0.0	525	1.5	540	3.6
V	0-1	511	16.5	526	17.0	541	15.1
	1-2	512	11.9	527	11.9	542	10.0
	2-3	513	10.7	528	10.0	543	7.7
	3-4	514	2.5	529	4.4	544	8.3
	4-6 2/3	515	0.3	530	1.5	545	4.4

[1] Blanks not deducted.

we find very little ammonia fixed in the upper few inches. The first and second inches have about the same, then there is a gradual increase in proportion to the depth. This curve in figure 5 is probably distorted from the natural one owing to the fact that the tube was so short. If the column of soil had been a foot or more in length it is reasonable to believe that approximately the same amount, 1.7 mg. of ammonia, would have been fixed per inch of depth; the reason that there is more ammonia present per inch in the lower layers is no doubt because it could not distribute itself at a greater depth.

In contrast to the behavior of Soil I, Soil II shows a strikingly large fixation of ammonia in the surface inch, almost two-thirds of the total amount applied being held there. Beyond the third inch in depth there is practically no further fixation. Since this soil is not heavy it perhaps is

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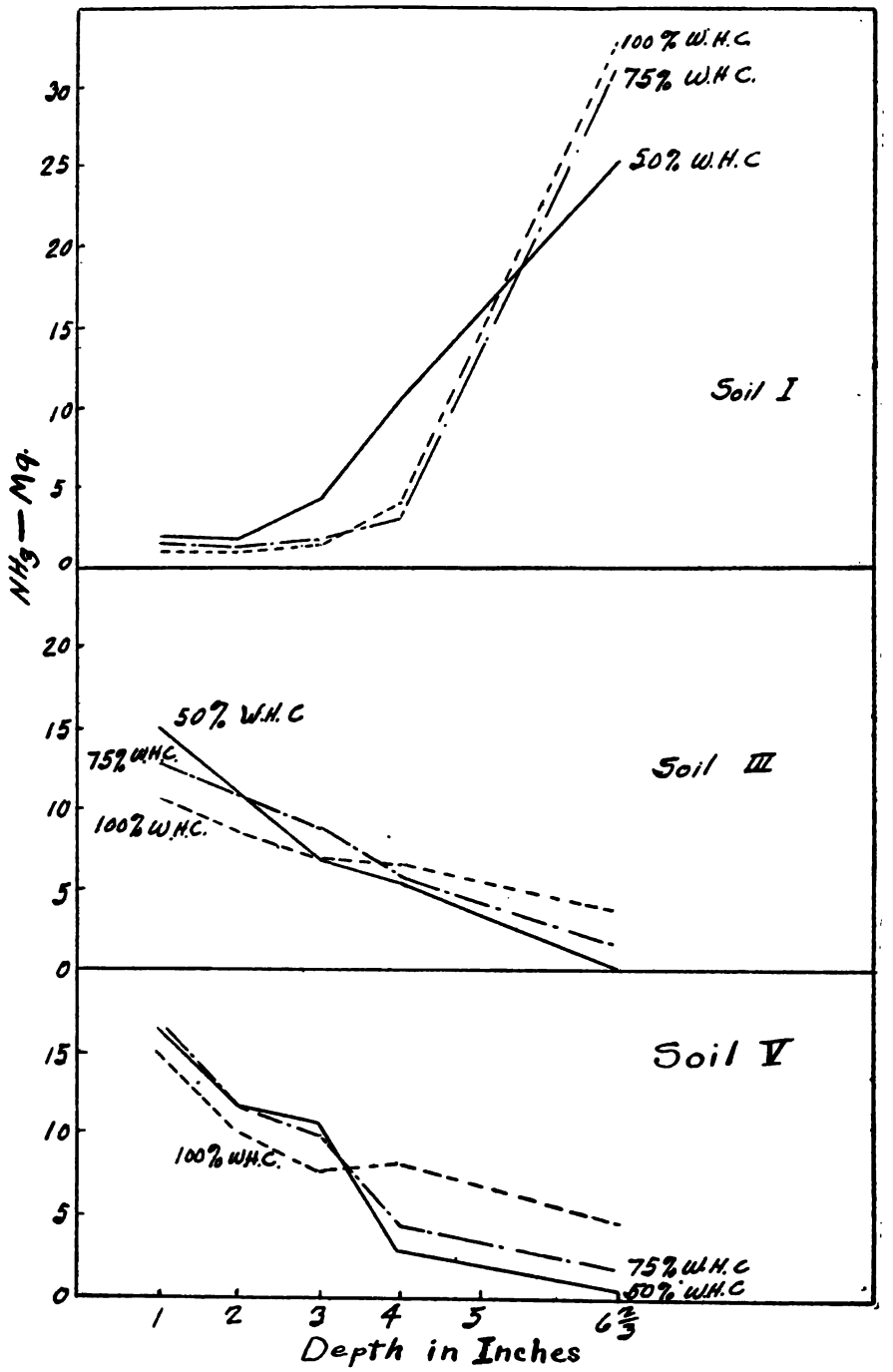

Fig. 4.—Diagram showing the effect of moisture upon the vertical distribution of ammonia.

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surprising that the fixing power is so great; however, it correlates very well with the behavior manifested in Part I.

With Soils III, IV, V, and VI we have practically the same conditions shown. Three of these soils show a maximum fixation in the first inch, and the larger amount in the second inch of Soil VI is very small. With this exception a gradual decrease with depth is observed in every case.

TABLE XXII

VERTICAL DISTRIBUTION OF AMMONIA

Soil No.	Lab. No.	Depth Inches	Ammonia		Soil No.	Lab. No.	Ammonia	
			Mg.	Av.Mg			Mg.	Av. Mg.
I	547	0-1	2.0	....	IV	583	11.4	....
	548	...	1.4	1.70		584	10.4	10.90
	549	1-2	2.0	....		585	8.2	....
	550	...	1.4	1.70		586	10.4	9.30
	551	2-3	3.4	....		587	8.3	....
	552	...	2.0	2.70		588	7.7	8.00
	553	3-4	5.1	....		589	5.8	....
	554	...	3.9	4.50		590	4.4	5.10
	555	4-5	10.1	....		591	2.5	....
	556	...	12.5	11.30		592	1.9	2.20
	557	5-6 2/3	18.1	....		593	0.9	....
	558	...	18.7	18.40		594	0.5	0.70
II	559	0-1	26.4	....	V	595	16.0	....
	560	...	30.4	28.40		596	16.8	16.40
	561	1-2	11.9	....		597	12.6	.. ..
	562	...	10.9	11.40		598	13.2	12.90
	563	2-3	4.9	....		599	6.6	....
	564	...	1.7	3.30		600	8.4	7.50
	565	3-4	0.9	....		601	1.5	....
	566	...	0.9	0.90		602	1.2	1.35
	567	4-5	0.5	....		603	0.5	....
	568	...	0.3	0.40		604	0.5	0.50
	569	5-6 2/3	—0.2	....		605	0.2	....
	570	...	—0.3	—0.25		606	0.2	0.20
III	571	0-1	15.1	....	VI	607	11.9	....
	572	...	13.8	14.45		608	9.2	10.55
	573	1-2	10.9	....		609	7.6	....
	574	...	10.7	10.80		610	13.8	10.70
	575	2-3	8.8	....		611	8.5	....
	576	...	8.4	8.60		612	5.8	7.15
	577	3-4	4.8	....		613	5.8	....
	578	...	5.3	5.05		614	6.3	6.05
	579	4-5	1.4	....		615	2.0	....
	580	...	1.9	1.65		616	5.1	3.55
	581	5-6 2/3	—0.3	....		617	1.3	....
	582	...	—0.2	—0.25		618	1.2	1.25

If we arrange the soils in Table XXII in the order of the amount of ammonia held in the first inch we have them in the following order: II, V, III, IV, VI, I, which it is interesting to note, corresponds exactly with their relative absorptive power for ammonia. We are without doubt warranted in believing that the greater a soil's absorptive capacity for ammonia, the nearer to the surface will the applied ammonia be fixed. And since the nearer the surface that the ammonia remains the less is the

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likelihood of loss, it perhaps is of some importance not to lose sight of at least one method of increasing the absorptive power of the soil.

As indicated in Table X, the use of CaO considerably increased the absorptive powers of all soils but one, which already had an excess of it. We would expect therefore, that the addition of lime to all soils but No. II, would effect a greater surface retention than is manifest in the untreated soils. Five-tenths-gram portions of CaO were accordingly weighed out, applied to the tubes containing the soils, and with a spatula mixed with the surface two inches of soil. Ammonia was added as before with moisture, and determined in the different sections after standing for two days.

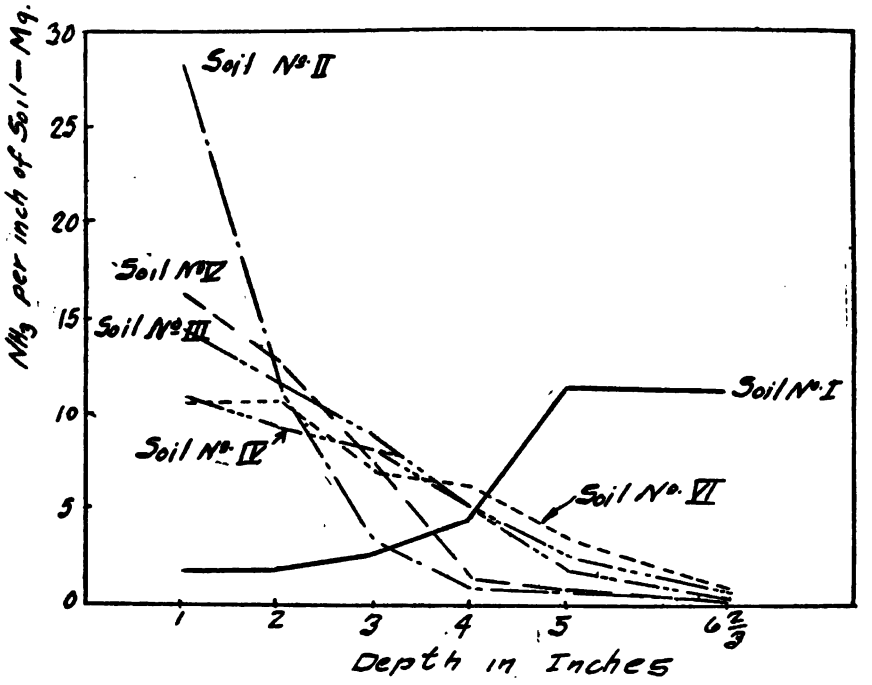

Fig. 5.—Diagram showing the vertical distribution of ammonia applied to soils.

In Table XXIII the effect of this treatment is fully brought out. In every instance but that of No. IV there is a decrease in the fixing power of the surface inch. At first this seems to be rather contrary to the effect which we would expect from Table X; however, if we pass to Table XI we can readily supply an explanation. The addition of 0.5 gm. CaO was apparently enough to produce a large excess in all but Soil IV. It will be remembered that this soil possessed the highest lime requirement and that in Table XI no appreciable depression was produced up to above 0.3 gm.

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per 50 gm. of soil. And since there was undoubtedly an excess in all the rest of the soils the lowering of the absorptive power is not strange. But in the second inch it is possible that not so much lime was present and the absorptive power was raised in every case. In the third inch there was with Soils I, II and VI an increase due to CaO, but a decrease with the

TABLE XXIII

EFFECT OF CaO UPON THE VERTICAL DISTRIBUTION OF AMMONIA

Soil	Lab	Depth Inches	0.5 gm. CaO Ammonia		Check Table XXII Av. Mg.	Increase due to CaO Av. Mg.	Soil No.	Lab. No.	0.5 gm. CaO Ammonia		Check Table XXII Av. Mg.	Increase due to Treatment. Av. Mg.
			Mg.	Av. Mg.					Mg.	Av. Mg.		
I	619	0-1	0.5	....	....	....	IV	655	13.9	....	....	....
	620	...	1.2	0.85	1.70	—0.85		656	13.4	13.65	10.90	2.75
	621	1-2	2.7	....	....	....		657	14.8	....	....	....
	622	...	2.6	2.65	1.70	0.95		658	11.5	13.15	9.30	3.85
	623	2-3	11.5	....	....	....		659	6.3	....	....	....
	624	...	8.8	10.15	2.70	7.45		660	3.4	4.85	8.00	—3.15
	625	3-4	11.0	....	....	....		661	1.7	....	....	....
	626	...	6.6	8.80	4.50	4.30		662	3.7	2.70	5.10	—2.40
	627	4-5	7.3	....	....	....		663	0.7	....	....	....
	628	...	8.3	6.95	11.30	—4.35		664	1.7	1.20	2.20	—1.00
	629	5-6 2/3	4.8	....	....	....		665	0.3	....	....	....
	630	...	11.7	8.25	18.40	—10.15		666	—0.2	0.05	0.70	—0.65
II	631	0-1	3.7	....	....	....	V	667	12.7	....	....	....
	632	...	4.3	4.00	28.40	—24.40		668	12.2	12.45	16.40	—3.95
	633	1-2	16.8	....	....	....		669	21.6	....	....	....
	634	...	17.4	17.10	11.40	5.70		670	20.2	20.90	12.90	8.00
	635	2-3	17.5	....	....	....		671	5.1	....	....	....
	636	...	18.2	17.85	3.30	14.55		672	6.8	5.95	7.50	—1.55
	637	3-4	2.4	....	....	....		673	1.0	....	....	....
	638	...	0.9	1.65	0.90	0.75		674	1.0	1.00	1.35	—0.35
	639	4-5	1.0	....	....	....		675	0.9	....	....	....
	640	...	0.2	0.60	0.40	0.20		676	0.3	0.60	0.50	0.10
	641	5-6 2/3	0.3	....	....	....		677	0.5	....	....	....
	642	...	—0.3	0.00	—0.25	—0.25		678	0.0	0.25	0.20	0.05
III	643	0-1	8.3	....	....	....	VI	679	5.1	....	....	....
	644	...	7.6	13.40	14.45	—1.05		680	2.7	3.90	10.55	—6.65
	645	1-2	19.2	....	....	....		681	12.4	....	....	....
	646	...	17.9	18.55	10.80	7.75		682	19.0	15.70	10.70	5.00
	647	2-3	7.1	....	....	....		683	13.8	....	....	....
	648	...	7.1	7.10	8.60	—1.50		684	9.5	11.65	7.15	4.50
	649	3-4	2.9	....	....	....		685	3.6	....	....	....
	650	...	4.9	3.90	5.05	—1.15		686	3.4	3.50	6.05	—2.55
	651	4-5	0.3	....	....	....		687	0.9	....	....	....
	652	...	0.2	0.25	1.65	—1.40		688	0.2	0.55	3.55	—3.00
	653	5-6 2/3	—0.3	....	....	....		689	0.0	....	....	....
	654	...	0.0	—0.15	—0.25	—0.40		690	—0.2	—0.10	1.25	—1.35

rest. Wherever a decrease was observed at this depth it is to be noted that there was a very large fixation in the upper two inches. There would be a very small amount of ammonia left to get down to the deeper layers and we would expect just the conditions shown. A point of interest is that of Soil II. The first inch held relatively little ammonia and the sec-

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ond, probably containing a little less CaO, held considerably more. At the third inch where the absorptive capacity of the soil was not impaired by the lime we get practically all the rest of the ammonia.

TABLE XXIV

EFFECT OF $CaCO_3$ UPON THE VERTICAL DISTRIBUTION OF AMMONIA

Soil No.	Lab. No.	Depth Inches	1 gm. $CaCO_3$ Ammonia Mg.	1 gm. $CaCO_3$ Ammonia Av. Mg.	Check Table XXII Av. Mg.	Increase due to $CaCO_3$ Av. Mg.	Soil No.	Lab. No.	1 gm. $CaCO_3$ Ammonia Mg.	1 gm. $CaCO_3$ Ammonia Av. Mg	Check Table XXII Av. Mg.	Increase due to $CaCO_3$ Av. Mg.
I	691	0-1	2.7	....	....	....	IV	727	10.2	....	....	....
	692	...	2.0	2.35	1.70	0.65		728	9.9	10.05	10.90	—0.85
	693	1-2	3.2	....	....	....		729	9.2	....	....	....
	694	...	3.6	3.40	1.70	1.70		730	9.2	9.20	9.30	—0.10
	695	2-3	2.4	....	....	....		731	6.6	....	....	....
	696	...	2.9	2.65	2.70	—0.05		732	5.1	5.85	8.00	—2.15
	697	3-4	3.2	....	....	....		733	6.8	....	....	....
	698	...	4.9	4.05	4.50	—0.45		734	6.5	6.65	5.10	1.55
	699	4-5	8.5	....	....	....		735	3.1	....	....	....
	700	...	9.9	9.20	11.30	—2.10		736	4.3	3.70	2.20	1.50
	701	5-6 2/3	21.2	....	....	....		737	—0.2	....	....	....
	702	...	17.7	19.45	18.40	1.05		738	—0.3	—0.25	0.70	—0.95
II	703	0-1	25.5	....	....	....	V	739	15.6	....	....	....
	704	...	25.5	25.50	28.40	—2.90		740	15.6	15.60	16.4	—0.80
	705	1-2	12.4	....	....	....		741	13.6	....	....	....
	706	...	13.8	13.10	11.40	1.70		742	13.8	13.70	12.90	0.80
	707	2-3	3.2	....	....	....		743	9.7	....	....	....
	708	...	2.0	2.60	3.30	—0.70		744	8.8	9.25	7.50	1.75
	709	3-4	0.3	....	....	....		745	3.1	....	....	....
	710	...	0.3	0.30	0.90	—0.60		746	2.9	3.00	1.35	1.65
	711	4-5	0.5	....	....	....		747	0.0	....	....	....
	712	...	0.3	0.40	0.40	0.00		748	0.5	0.25	0.50	—0.25
	713	5-6 2/3	0.3	....	....	....		749	—0.5	....	....	....
	714	...	0.0	0.15	—0.25	0.40		750	0.3	—0.10	0.20	—0.30
III	715	0-1	11.7	....	....	....	VI	751	10.0	....	....	....
	716	...	11.2	11.45	14.45	—3.00		752	7.7	8.85	10.55	—1.70
	717	1-2	10.7	....	....	....		753	9.2	....	....	....
	718	...	10.9	10.80	10.80	0.00		754	8.5	8.85	10.70	—1.85
	719	2-3	8.0	....	....	....		755	5.3	....	....	....
	720	...	6.8	7.40	8.60	—1.20		756	4.3	4.80	7.15	—2.35
	721	3-4	7.3	....	....	....		757	8.0	....	....	....
	722	...	7.7	7.50	5.05	2.45		758	8.7	8.35	6.05	2.30
	723	4-5	1.7	....	....	....		759	3.7	....	....	....
	724	...	3.2	2.45	1.65	0.80		760	5.8	4.75	3.55	1.20
	725	5-6 2/3	0.0	....	....	....		761	0.2	....	....	....
	726	...	0.3	0.15	—0.25	0.40		762	0.2	0.20	1.25	—1.45

It will be noted throughout that the tendency of the lime is to locate most of the ammonia in the second and third inches of the soil where we perhaps have the most efficient bacterial activities. In practically every case a larger amount of ammonia is held in the first three inches in the limed than in the unlimed soils.

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That the effect of $CaCO_3$ upon the vertical distribution is of no great importance is shown in Table XXIV. If anything the tendency is toward lowering slightly the position of the ammonia. Why this should be true seems inexplicable with the data at hand.

TABLE XXV

EFFECT OF K_2SO_4 UPON THE VERTICAL DISTRIBUTION OF AMMONIA

Soil No.	Lab. No.	Depth Inches	0.25 gm. K_2SO_4 Ammonia		Check Table XXII Av. Mg.	Increase due to K_2SO_4 Av. Mg.	Soil No.	Lab. No.	0.25 gm. K_2SO_4 Ammonia		Check Table XXII Av. Mg.	Increase due to K_2SO_4 Av. Mg.
			Mg.	Av. Mg.					Mg.	Av. Mg.		
I	763	0-1	1.9	....	....	....	IV	799	11.4	....	....	....
	764	...	1.4	1.65	1.70	—0.05		800	7.3	9.35	10.90	—1.55
	765	1-2	1.5	....	....	....		801	6.5	....	....	....
	766	...	1.5	1.50	1.70	—0.20		802	8.2	7.35	9.30	—1.95
	767	2-3	1.9	....	....	....		803	7.1	....	....	....
	768	...	1.5	1.70	2.70	—1.00		804	6.1	6.60	8.00	—1.40
	769	3-4	3.1	....	....	....		805	9.0	....	....	....
	770	...	2.4	2.75	4.50	—1.75		806	7.7	8.35	5.10	3.25
	771	4-5	8.3	....	....	....		807	1.5	....	....	....
	772	...	7.0	7.65	11.30	—3.65		808	8.5	5.00	2.20	2.80
	773	5-6 2/3	24.8	....	....	....		809	3.4	....	....	....
	774	...	26.7	25.75	18.40	7.35		810	3.6	3.50	0.70	2.80
II	775	0-1	24.8	....	....	....	V	811	14.6	....	....	....
	776	...	21.2	23.00	28.40	—5.40		812	14.0	14.30	16.40	—2.10
	777	1-2	12.7	....	....	....		813	12.1	....	....	....
	778	...	14.5	13.60	11.40	2.20		814	11.6	11.85	12.90	—1.05
	779	2-3	4.3	....	....	....		815	10.4	....	....	....
	780	...	5.4	4 .85	3.30	1.55		816	12.4	11.40	7.50	3.90
	781	3-4	1.4	....	....	....		817	4.9	....	....	....
	782	...	1.0	1.20	0.90	0.30		818	7.0	5.95	1.35	4.60
	783	4-5	0.3	....	....	....		819	0.3	....	....	....
	784	...	0.2	0.25	0.40	—0.15		820	1.2	0.75	0.50	0.25
	785	5-6 2/3	0.3	....	....	....		821	0.5	....	....	....
	786	...	0.0	0.15	—0.25	0.40		822	0.2	0.35	0.20	0.15
III	787	0-1	14.3	....	....	....	VI	823	9.7	....	....	....
	788	...	10.9	12.60	14.45	—1.85		824	8.8	9.25	10.55	—1.30
	789	1-2	7.2	....	....	....		825	6.6	....	....	....
	790	...	8.5	8.85	10.80	—1.95		826	6.1	6.35	10.70	—4.35
	791	2-3	6.6	....	....	....		827	6.8	....	....	....
	792	...	7.3	6.95	8.60	—1.65		829	6.3	6.55	7.15	—0.50
	793	3-4	8.2	....	....	....		829	6.3	....	....	....
	794	...	7.1	7.65	5.05	2.60		830	8.2	7.25	6.05	1.20
	795	4-5	6.5	....	....	....		831	8.0	....	....	....
	796	...	6.5	6.50	1.65	4.85		832	6.1	7.05	3.55	3.50
	797	5-6 2/3	0.5	....	....	....		833	2.7	....	....	....
	798	...	1.9	1.20	—0.25	1.45		834	2.4	2.55	1.25	1.30

When ammonium sulfate is applied to soils with either sulfate of potash or acid phosphate a modification of the distribution will be effected. In order to study these modifications 0.25-gm. portions of K_2SO_4 were added to one set of soils in tubes, and 0.25-gm. portions of acid phosphate to another. The effect of these two materials upon the distribution of

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ammonia was determined and recorded in Tables XXV and XXVI.

Since potassium is able to replace ammonium it would appear that the effect of adding it would be to lower the absorption of ammonia in the surface layers of the soil. A study of the table shows this to be exactly

TABLE XXVI

EFFECT OF ACID PHOSPHATE UPON THE VERTICAL DISTRIBUTION OF AMMONIA

Soil No.	Lab. No.	Depth Inches	0.25 gm. Acid Phos. Ammonia		Check Table XXII Av. Mg.	Increase due to A. P. Av. Mg.	Soil No.	Lab. No.	0.25 gm. Acid Phos. Ammonia		Check Table XXII Av. Mg.	Increase due to A. P. Av. Mg.
			Mg.	Av. Mg.					Mg.	Av. Mg.		
I	835	0-1	1.9	....	....	....	IV	871	8.0	....	....	....
	836	...	0.7	1.30	1.70	—0.40		872	5.4	6.70	10.90	—4.20
	837	1-2	1.9	....	....	....		873	6.8	....	....	....
	838	...	1.4	1.65	1.70	—0.05		874	6.5	6.65	9.30	—2.65
	839	2-3	2.0	....	....	....		875	6.1	....	....	....
	840	...	2.4	2.20	2.70	—0.50		876	7.8	6.95	8.00	—1.05
	841	3-4	3.1	....	....	....		877	9.3	....	....	....
	842	...	5.5	4.30	4.50	—0.20		878	8.3	8.80	5.10	3.70
	843	4-5	7.8	....	....	....		879	8.5	....	....	....
	844	...	10.5	9.15	11.30	—2.15		880	4.8	6.65	2.20	4.45
	845	5-6 2/3	25.2	....	....	....		881	1.7	....	....	....
	846	...	18.9	22.05	18.40	3.65		882	—0.2	0.75	0.70	0.05
II	847	0-1	21.6	....	....	....	V	883	12.7	....	....	....
	848	...	24.5	23.05	28.40	—5.35		884	12.2	12.45	16.40	—3.95
	849	1-2	12.9	....	....	....		885	9.0	....	....	....
	850	...	13.4	13.15	11.40	1.75		886	13.4	11.20	12.90	—1.70
	851	2-3	5.1	....	....	....		887	10.4	....	....	....
	852	...	4.3	4.70	3.30	1.40		888	10.9	10.65	7.50	3.15
	853	3-4	1.2	....	....	....		889	9.0	....	....	....
	854	...	—0.2	0.50	0.90	—0.40		890	3.1	6.05	1.35	4.70
	855	4-5	0.7	....	....	....		891	2.2	....	....	....
	856	...	0.3	0.50	0.40	0.10		892	0.3	1.25	0.50	0.75
	857	5-6 2/3	0.9	....	.. ..	....		893	0.5	....	....	....
	858	...	0.0	0.45	—0.25	0.70		894	0.0	0.25	0.20	0.05
III	859	0-1	12.7	....	....	....	VI	895	9.4	....	....	....
	860	...	9.0	10.85	14.45	—3.60		896	5.1	7.25	10.55	—3.30
	861	1-2	7.3	....	....	....		897	6.3	....	....	....
	862	...	10.7	9.00	10.80	—1.80		898	5.4	5.85	10.70	—4.85
	863	2-3	5.8	....	....	....		899	7.0	....	....	....
	864	...	10.2	8.00	8.60	—0.60		900	8.7	7.85	7.15	0.70
	865	3-4	8.3	....	....	....		901	7.0	....	....	....
	866	...	8.0	8.15	5.05	3.10		902	10.4	8.70	6.05	2.65
	867	4-5	6.6	....	....	....		903	7.7	....	....	....
	868	...	0.7	3.65	1.65	2.00		904	4.4	6.05	3.55	2.50
	869	5-6 2/3	0.5	....	....	....		905	2.6	....	....	....
	870	...	—0.3	0.10	—0.25	0.35		906	—0.5	1.05	1.25	—0.20

the case. There is an increase in the amount of ammonia in the lower layers at the expense of the surface layers. It is doubtful if this would have any practical significance in any but the lightest soils where the danger of loss by leaching is at a maximum.

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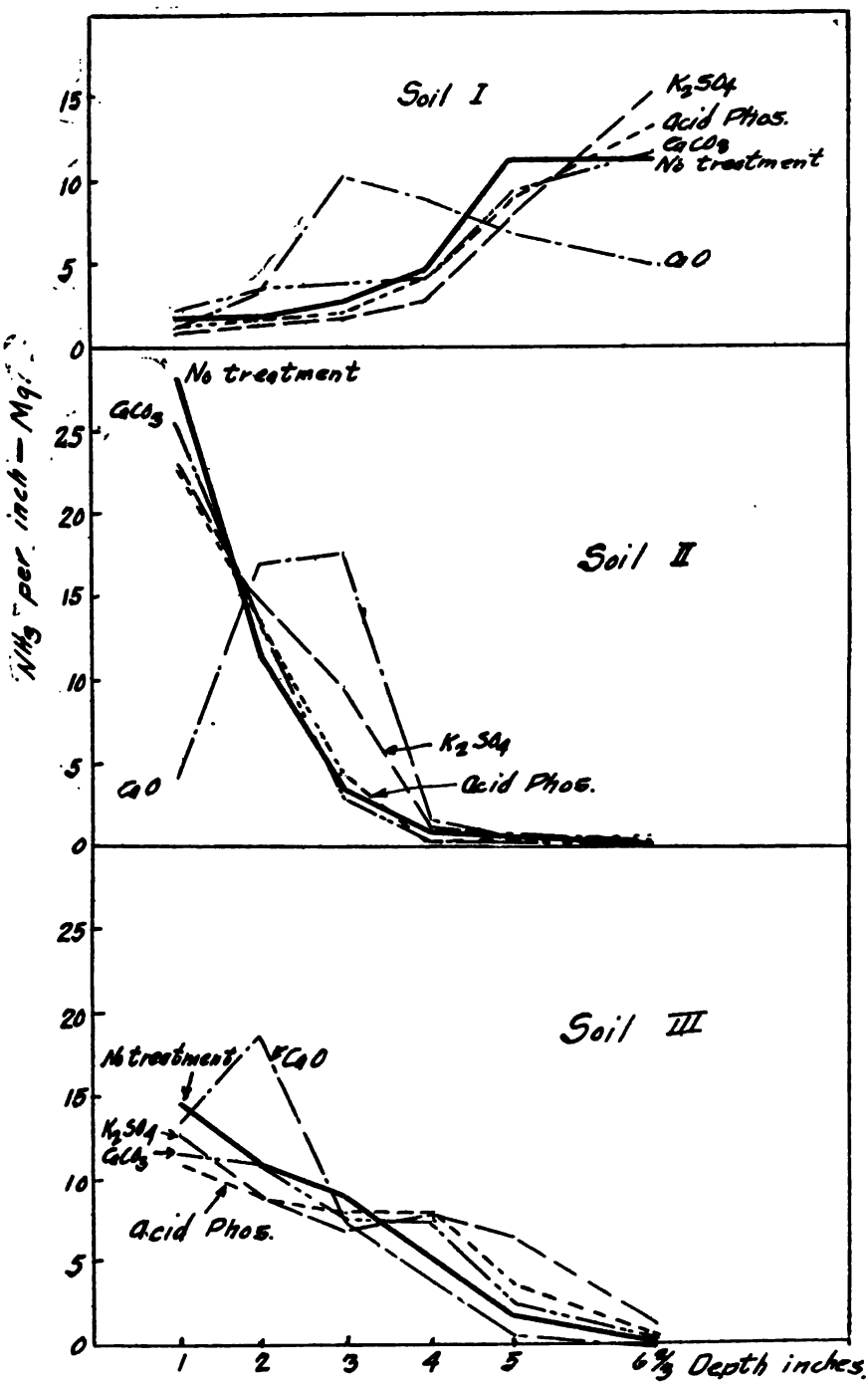

Fig. 6.—Diagram showing the effect of CaO, CaO_3, K_2SO_4 and acid phosphate upon the vertical distribution of applied ammonia (Soils I, II and III).

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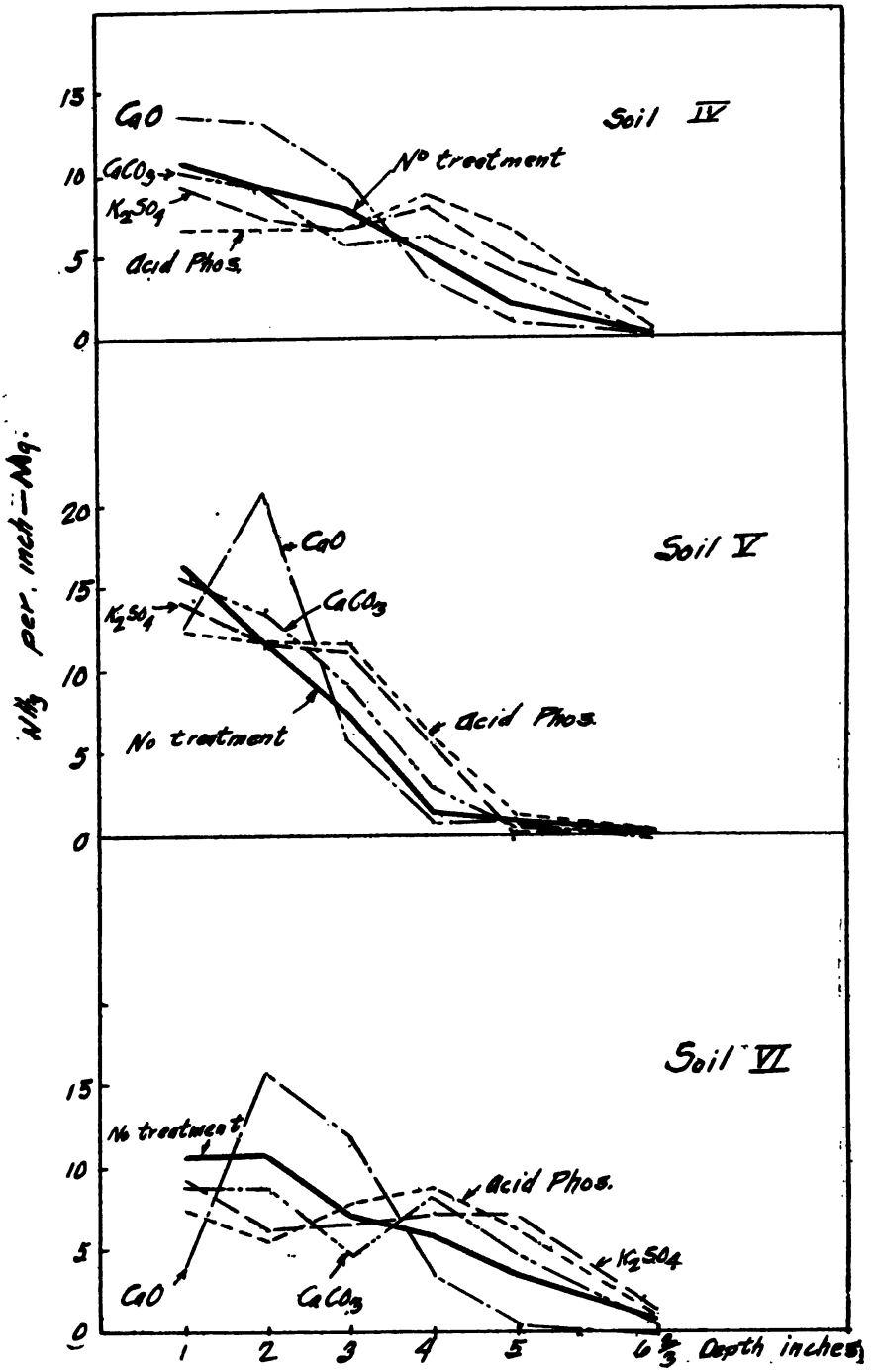

Fig. 7.—Diagram showing the effect of CaO, $CaCO_3$, K_2SO_4 and acid phosphate upon the vertical distribution of applied ammonia (Soils IV, V and VI).

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The effect of acid phosphate as seen in Table XXVI seems to be greater than that of potash per unit applied, and since there is usually a much larger amount of the former applied per acre it is possible that its effect may reach significant proportions. The use of both acid phosphate and potash with ammonia may have their action magnified as indicated by Table XIX. Even if there were no danger of direct loss from this cause it would undoubtedly have a deleterious influence in most cases due to the lowering of the absorbed ammonia.

The effects of CaO, $CaCO_3$, K_2SO_4 and acid phosphate upon the vertical distribution of applied ammonia are graphically represented in figures 6 and 7.

It appears evident that the distribution of ammonia applied to soils is a variable having wide limits depending largely upon the physical nature of the soils but also appreciably upon their chemical constitution. The presence of lime up to a certain point will enhance the proper distribution and in a practical way will undoubtedly tend toward conserving the nitrogen applied. Acid phosphate and potash exert an influence which might not be considered beneficial to the best distribution. It is, however, probable that the effect of these elements in other ways, principally biologically, serves to more than compensate for this, and makes their resultant effect decidedly advantageous in the utilization of nitrogen from ammonium sulfate.

To give to the results a practical interpretation requires considerable caution. In the absence of much experimental data concerning the method of application of sulfate of ammonia, it is perhaps necessary to apply the results obtained in a more or less flexible way. Nevertheless, certain points were brought out in regard to the distribution of the applied ammonia which will undoubtedly help in our understanding of the problems involved.

The indications therefrom are that ammonium sulfate should be applied much shallower in light than in heavy soils, particularly if the soils are acid. The presence of burnt lime is preferable to ground limestone if the danger of loss of nitrogen by leaching is to be considered. The use of potash and acid phosphate would argue for a shallower application of ammonia.

Summary

1. New Jersey soils present wide ranges of absorptive capacity for ammonia; the smallest amount was observed in the lightest soil, the Norfolk sand, corresponding to 266 pounds of ammonium sulfate per acre 6 2/3 inches; the largest amount in the Collington sandy loam of 2642 pounds per acre.

2. That the absorption process is a very complicated one, depending upon many factors and not the result of any one or two, appears to be

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beyond doubt. For any one factor, the presence of CaO seems to exert more influence than any other. In general, mechanical and chemical composition, hygroscopity, etc., all undoubtedly have some effect; to a certain extent, the absorption of ammonia follows the percentage of combined iron and alumina, calcium oxide and carbon dioxide, as well as organic matter and physical properties of the soil. The presence of colloids, and conditions which favor them increase the absorptive power of the soil. Soils owe their property of absorption of ammonia to chemical and physical factors, and perhaps others.

3. The presence of potash or acid phosphate, either alone or in combination, reduces the absorptive capacity of the soil.

4. The distribution of applied ammonia in soils is also quite variable and seems to be a function of the absorption. In light soils the ammonia quickly finds its way into the deeper layers unless there is a supply of CaO present; in heavier soils, most of the ammonia is held in the surface three or four inches. A slightly alkaline reaction is beneficial to the conservation of ammonia. Calcium carbonate has little effect upon the distribution of ammonia in soils. Everything else being equal, ammonium sulfate should be applied shallower in light than in heavy soils.

5. Potash or acid phosphate tends to lower the mean distribution of the ammonia.

6. A correlation exists between the absorptive capacity of a soil for ammonia and the depth of its distribution; the greater this capacity the nearer the surface will it remain.

Acknowledgment

The author desires to express his appreciation and gratitude to Mr. C. G. Atwater, Prof. A. W. Blair, and particularly to Dr. J. G. Lipman, for aid and suggestions in the planning and carrying out of the above work.

Literature Cited

(1) Aberson, J. H.
1911. The absorption phenomena of cultivated soils. *In* Meded. Rijks. Hovgere Land, Tuin en Boschbouwsh. [Wageningen] Bd. 5, No. 1, p. 1-43 Abs. *in* Jour. Soc. Chem. Indus., v. 31 (1912), no. 5, p. 243.

(2) Armsby, H. P.
1877. On the absorption of bases by the soil. *In* Amer. Jour. Sci., s. 3, v. 14, (114), p. 25-30.

(3) Biedermann, R.
1869. Einige Beiträge zu der Frage der Bodenabsorption. *In* Landw. Vers. Stat., Bd. 11, p. 1-69, 81-95.

(4) Blanck, E.
1907. On lime concretions. *In* Landw. Vers. Stat., Bd. 65, no. 5-6, p. 471-479. Abs. *in* Jour. Chem. Soc. [London], v. 92, no. 534, II, p. 295; Exp. Sta. Rec., v. 18, p. 1024.

Digitized by Google

(5) CAMERON, F. K.
1911. The Soil Solution, p. 60. Easton, Pa.

(6) CRAWLEY, J. T., and DUNCAN, R. A.
1903. On the fixation of ammonia and potash by Hawaiian soils. *In* Jour. Amer. Chem. Soc., v. 25, no. 1, p. 47-50.

(7) DITTRICH, M.
1901. Chemical-geological investigations on the absorptive properties of decomposed rocks. *In* Mitt. Grossherz. Bad. Geol. Landes-Aufnahme, Bd. 4, No. 3. Abs. *in* Centbl. Agr. Chem., Bd. 31, No. 12, p. 793-796; Exp. Sta. Rec., v. 14, p. 746.

(8) EICHORN, H.
1858. Ueber die Einwirkung verdünnter Salzlösungen auf Ackererde. *In* Landw. Centbl. Posen, Jahrg. 6, Bd. 2, p. 169-175.

(9) ENGLES, O.
1911. Absorptive capacity of some Palatinate soils for different plant nutrients. *In* Landw. Jahrb. Bayern., Bd. 1, No. 9, p. 689-706.

(10) GANS, R.
1913. The chemical and physical nature of colloidal aluminum silicates containing water. *In* Centbl. Min. Geol. u. Paläontol., No. 9, p. 273-279; No. 10, p. 299-306; No. 22, p. 699-712; No. 23, p. 728-741.

(11) HALL, A. D., and GIMINGHAM, C. T.
1907. The interaction of ammonium salts and the constituents of the soil. *In* Jour. Chem. Soc. [London], v. 91-92, no. 534, p. 677-687.

(12) HEIDEN, E.
1864. Ein Beitrag zur Erklarung der Ursachen der Absorption. *In* Ann. Landw. Königl. Preuss. Staaten, Bd. 43, p. 310-326.

(13) HUSTON, H. A., and GOSS, A.
1890. The absorptive power of soils. *In* Purdue Univ. Agr. Exp. Sta. Bul. 33, p. 46-54.

(14) KARPIZOV, K. S.
1904. On the absorptive capacity of different layers of soils. *In* Pochovovyedenie (Pédologie), Bd. 6, No. 2, p. 137-151. Abs. *in* Exp. Sta. Rec., v. 16, p. 652.

(15) KNOP, W.
1871. Die Bonitirung der Ackererde, p. 49. Leipzig.

(16) LEMMERMANN, O., and FRESENIUS, L.
1913. Increasing the ammonia-fixing power of soils. *In* Fühling's Landw. Ztg., Bd. 61, No. 7, p. 240-253; No. 8, p. 274-285.

(17) MCGEORGE, WM.
1914. Absorption of fertilizer salts by Hawaiian soils. Hawaii Agr. Exp. Sta. Bul. 35, 32 p.

(18) MITSCHERLICH, E. A.
1914. Solution and absorption in the soil. *In* Landw. Jahrb., Bd. 46, No. 3, p. 413-430.

(19) MORSE, F. W., and CURRY, B. E.
1908. Reactions between manurial salts and clays, mucks and soils. N. H. Agr. Exp. Sta. 29th Ann. Rpt., p. 271-293.

Digitized by Google

(20) PATTEN, H. E., and WAGGAMAN, W. H.
1908. Absorption by soils. U. S. Dept. Agr. Bur. Soils Bul. 52.

(21) PRATOLONGO, U.
1910. The absorptive capacity of soils from the physico-chemical standpoint. *In* R. Ist. Lombardo Sci. e Let. Rend., s. 2, v. 43, no. 16, p. 542-555.

(22) RAUTENBERG, F.
1862. Ueber die Absorptionsfähigkeit verschiedener Bodenarten und das geognostische Vorkommen derselben. *In* Jour. Landw., n. F., Bd. 7, p. 49-66.

(23) ROHLAND, P.
1910. Colloidal materials in clay and absorption phenomena. *In* Landw. Jahrb. Bd. 39, No. 3, p. 369-372. Abs. *in* Chem. Zentbl. (1910) II, No. 7, p. 491.

(24) RUPRECHT, R. W., and MORSE, F. W.
1915. Effect of sulfate of ammonia on soil. Mass. Agr. Exp. Sta. Bul. 165.

(25) SABANIN, A. N.
1908. On the absorptive powers of some Russian soils. *In* Pochvovyedyenie (Pédologie), 1908, p. 87-98. Abs. *in* Exp. Sta. Rec., v. 22, p. 520.

(26) STOKLASA, J.
1912. The biological absorption of soils. *In* Chem. Ztg., Bd. 35, No. 154, p. 1425-1427.

(27) TADSKARO, T.
1914. Colloidal properties of acid soils of Japan. *In* Jour. Col. Agr. Tohoku Imp. Univ., v. 6, no. 2, p. 27-50.

(28) TUXEN, C. F. A.
1882. Untersuchungen über die Wirkung des Chilisalpeters, des Kochsalzes und des Chlorokaliums im Erdboden. *In* Landw. Vers. Stat., Bd. 27, p. 107-113.

(29) WARINGTON, JR., R.
1868. On the part taken by oxide of iron and alumina in the absorptive action of soils. *In* Jour. Chem. Soc. (London), n. s., v. 6, (21), p. 1-19.

(30) WAY, J. T.
1850. On the power of soils to absorb manure. *In* Jour. Roy. Agr. Soc. England, v. 11, p. 313-379.

(31) WHITNEY, M.
1891. Soil investigations: IX—The circulation of water in type soils. *In* Md. Agr. Exp. Sta., 4th Ann. Rpt., p. 282-286. Also 1892. Some physical properties of soils in their relations to moisture and crop distribution. U. S. Dept. Agr. Wea. Bur. Bul. 4, 90 p.

(32) WIEGNER, G.
1912. Regarding the exchange of bases in cultivated soils. *In* Jour. Landw., Bd. 60, No. 2, p. 111-150; No. 3, p. 172-222. Abs. *in* Jour. Chem. Soc. (London), v. 102, no. 600, II, p. 891.

Digitized by Google

(33) WIEGNER, G.
1914. The chemical and physical nature of colloidal aluminum silicates containing water. *In* Centbl. Min., Geol. u. Paläontol., 1914, No. 9, p. 262-272.

(34) WILEY, H. W.
1906. Principles and Practice of Agricultural Analysis. Second Edition. v. 1, p. 129. Easton, Pa.

(35) ZHOLTINSKI, I. P.
1908. The absorptive capacity of some Russian soils and of their finest particles as related to their composition. *In* Zhur. Opuitn. Agron. (Russ. Jour. Expt. Landw), Bd. 9, No. 2, p. 129-225. Abs. in Exp. Sta. Rec., v. 20, p. 916.

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THE NITRIC NITROGEN CONTENT OF THE COUNTRY ROCK[1]

A Contribution Regarding the Origin of Nitre Spots in Certain Western Soils

By

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I. Historical

The progress of the work upon the general problem of the origin of the nitre spots in certain western soils has developed several different points of view. The fact that certain spots in western cultivated soils were unusually rich in nitrates was first observed by Hilgard (6, p. 68), who attributed their accumulation to the more rapid nitrification of the organic matter of the soil in the warm arid climate of the West when the moisture limit was removed by irrigation.

A number of years later an entirely different explanation was offered for a similar condition observed by Headden (3) in certain Colorado soils. Spots were observed on which nothing would grow and examination showed the soil to be rich in nitrates and also other alkali salts consisting of the chlorides and sulphates of calcium, magnesium, sodium, and potassium in varying amounts. Soil, which had been formerly productive and of great value, became non-productive and almost valueless. In many cases, valuable orchards were partially or entirely ruined by these accumulations. The nitrates present were attributed by Headden to the fixation of atmospheric nitrogen by the non-symbiotic bacteria. This conception has been further amplified by Headden (4, 5) and also by Sackett (7).

Our investigations (10; 12, p. 128) at the Utah Experiment Station, extending over a period of ten years, regarding the influence of irrigation water upon the production and movement of nitrates in irrigated soils, led us to an entirely different conception regarding the origin of these nitre spots. We had found that nitrification was not appreciably more rapid in arid soil than in the humid soil when measured by the nitrates

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present in the soil at various depths and in definite seasons throughout the year and from year to year. For example, Warrington (15, p. 82) found at Rothamsted after lucerne that there was 3.3 parts per million of nitric nitrogen; in Utah, Stewart and Greaves, found 0.8 parts per million in irrigated land and 1.8 parts per million in dry-farmed land. In wheat soil after clover, Warrington found at Rothamsted 2.2 parts per million; while in Utah, Stewart and Greaves found 0.8 parts in dry-farm land. After fallow Warrington found 1.3 parts of nitric nitrogen per million; while Stewart and Greaves in Utah found 5.4 parts per million. These facts, together with the additional fact that wherever Dr. Hilgard or Dr. Headden had reported the data from the nitre spots they also always reported the presence of additional amounts of other alkali salts, led us to suspect that the nitre spots were the direct result of the leaching and concentration of the nitrates by the movement of the ground water. This conclusion received considerable support when a close examination (11) of Dr. Headden's results showed clearly that the nitric nitrogen and chlorine *in any given nitre spot* varied in the same ratio from year to year or period to period within a year.

A preliminary investigation (8) showed that the country rock adjacent to the affected areas was impregnated with alkali salts including the nitrates. These results were so significant and important as to warrant continued additional work along the same lines. It seemed imperative that we should learn more of the geology of the affected areas. This study led to extensive investigations regarding the nitrate content of the country rock and the geological history of the area, the results of which have been partially published (13, 14).

The former work reported the results of investigations regarding the nitrate content of the country rock of eastern Utah, western Colorado and southwestern Wyoming, and consisted of a study of the Jurassic, Cretaceous and Tertiary deposits of shale, sandstone, gypsum, ash and rock salt. It was found that the shales and sandstones of the Cretaceous and Tertiary were heavily impregnated with nitrate accumulations. *All Cretaceous material was found to contain nitrate.* Cretaceous sandstone contained a maximum amount of 0.293 per cent sodium nitrate, the shale contained a maximum of 1.026 per cent; the clay 0.115 per cent, the ash[1] 3.270, while the surface alkali soil contained 3.35 per cent. The Tertiary sandstone contained a maximum amount of 0.284 per cent, the shale 0.750 per cent, the clay 0.940 per cent, the ash, 3.280 per cent, while the surface alkali soil contained 0.232 per cent. While the Tertiary material contained a slightly lower amount, the greater extent of exposed area belong to this series. This renders a great area of soil derived from the Tertiary rock

[1] Ash is a term used throughout this paper to indicate the mixture of dry fine clay and dry white crystals of alkali salts. This mixture is found over the clay hills just under a hard clay crust on the surface.

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subject to nitrate accumulation. The maximum, minimum, and average amounts of sodium nitrate in the three classes of material are reported in Table A.

The Jurassic deposits are not so heavily charged with alkali and nitrate accumulations as are those of the Cretaceous or Tertiary.

TABLE A

PER CENT SODIUM NITRATE IN CRETACEOUS MATERIAL

	Maximum	Minimum	Average
Sandstone	0.293	0.005	0.055
Shale	1.026	0.003	0.104
Clay	0.115	0.002	0.039
Ash	3.270	0.005	0.870
Alkali	3.350	0.003	0.074
TERTIARY			
Sandstone	0.284	None	0.074
Shale	0.750	None	0.147
Clay	0.940	None	0.053
Ash	3.280	0.0002	0.236
Alkali	0.232	None	0.021
JURASSIC			
Sandstone	0.039	None	0.008
Shale	0.019	None	0.005
Ash	0.202	0.149	0.175
Alkali	0.017	0.017	0.017

The data presented in this paper are the results of a continuation of the investigations upon the nitrate content of the country rock of other portions of the state of Utah, the deposits of which belong partially to other geological series than the Cretaceous, Tertiary or Jurassic. The rock investigated belongs in part to the Triassic, Permian and Pennsylvanian, but for convenience of discussion all material is grouped together from some central agricultural point.

II. Method of Investigation

The samples were all collected from the southwestern part of Utah or the northwestern part of Arizona in the manner already described. The collections were made in the summer of 1914. The nitric nitrogen and other alkali salts were determined in all the samples collected. The discussion of the significance of the alkali accumulations in the country rock is reserved for a future publication but for convenience the total amount of soluble salts is here reported together with the ratio of nitric nitrogen to the total salts present.

Fifty grams of the finely ground rock were placed in a large bottle with 1000 c.c. of distilled water and placed in a shaking machine for 8 hours. The solution was separated by filtration through a Chamberlain-Pasteur filter. The nitric nitrogen was determined in the soil extract by the aluminum reduction method.

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III. The Country Rock at St. George

The results of the analysis of the rock north of St. George, Utah, are reported in Table I, which for convenience of study is subdivided into four divisions. It may be seen from a study of the results presented that there are many times as much nitric nitrogen in the original rock near Santa Clara, southern Utah, as we found in the normal irrigated soils. Sample No. 323 contains over forty times as much as the maximum amount we found in our ordinary well cultivated soils.

Even the average amounts in eight samples of rock is sixteen times the maximum in productive soils of Utah.

TABLE I

NITRIC NITROGEN CONTENT OF THE COUNTRY ROCK NORTH OF ST. GEORGE, UTAH

Field No.	Description and location of samples	Total soluble salts p. p. m.	Nitric Nitrogen	
			Parts per million	Per cent of total salts
	A.—GYPSUM			
319	Gypsum a few feet from No. 318.....	34,270	76.1	0.220
318	Gypsum and saponite west of No. 317.....	54,520	40.9	0.071
317	Gypsum 1¼ miles east of Santa Clara, Utah...	47,360	35.1	0.074
	B.—SHALE FROM TYPICAL CLAY HILL			
323	Disintegrated shale and dust near top of the hill	20,000	210.6	1.05
321	The dark undecomposed shale.....	7,120	198.9	2.79
322	Darker shale higher up than No. 321.....	8,000	40.9	0.512
	C.—VOLCANIC ASH			
313	Volcanic ash, Diamond Valley	6,520	31.2	0.47
	D.—BAUXITE CLAYS			
320	Bauxite near Santa Clara.....	3,440	17.6	0.51
	General average	29,989	81.4	0.71

The nitrates, as in many of the nitre spots, constitute only a small part of the soluble salts present in the country rock. As an average only 0.7 per cent of the soluble material is nitric nitrogen. The average amount of nitric nitrogen present is 81.4 parts per million while there is an average of 29,989 parts per million of total alkali salts.

The gypsum deposits as represented by Nos. 319, 318 and 317 contain large quantities of material soluble in water, an appreciable quantity of which is nitrate. The quantity is not large when considered from a commercial point of view, but when the immense quantity of the gypsum deposits is considered and their bearing on the material contributed to the

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soil formation, the amount of nitrate present in these deposits is of considerable significance.

The samples of shale, Nos. 323, 321 and 322, were collected from a typical clay hill on which no vegetation was growing. These clay hills, "on which nothing will grow," are characteristic of many areas of southern Utah and western Colorado. And it is important to consider them very carefully in seeking a solution of the origin of the nitre spots.

This particular clay hill is fully as characteristic as those occurring in eastern Utah which have already been described. The characteristic ash-like condition was observed on the sides and top of the hill. Both the shale and ash material are rich in nitrate and water-soluble salts, although not so rich as some of those observed in eastern Utah. A photograph was taken of this hill and is reproduced in Plate I, figure I.

No. 313, a good representative of the extensive volcanic ash deposits of southern Utah, contains an appreciable amount of soluble salts which contain some nitrate.

TABLE II

NITRIC NITROGEN CONTENT OF THE COUNTRY ROCK WEST OF ST. GEORGE, UTAH

Field No.	Description and location of samples	Total soluble salts p. p. m.	Nitric Nitrogen	
			Parts per million	Per cent of total salts
331	White shale 2 miles above No. 330.....	41,040	450.0	1.10
330	Shale 30 feet below No. 329.....	1,480	356.0	24.11
329	Shale 8 miles below St. George.....	5,480	216.0	3.99
326	Red and gray shale on banks of Rio Virgin....	3,880	134.0	3.46
332	Shale and sandstone.....	3,920	35.0	0.89
327	Alkali salts near No. 326.....	138,600	23.0	0.16
334	Ash-like soil underneath the clay.....	115,000	23.0	0.02
333	Sample of clay.....	82,300	12.0	0.014
	Average	48,962	156.0	4.22

Eight samples of shale, alkali and clay were collected from the deposits west of St. George, Utah, down the valley of the Rio Virgin River. These deposits probably belong to the lower Triassic or upper Permian series. The results are reported in Table II. All of the material is rich in nitrates and other water-soluble salts. Sample 331, undecomposed shale, contains over 80 times as much nitric nitrogen as reported in our best agricultural soils either in Utah or at Rothamsted. The average amount present in all the material is over 30 times this amount. These facts emphasize the significance of the nitrates present in the undecomposed rock of large areas of the Arid West.

Ten samples of material were collected east of St. George up the valley of the Rio Virgin River. The samples were taken as we ascended in the Triassic series of deposits. These results are recorded in Table III.

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Again there is a large quantity of soluble salts, part of which is nitrate; the nitrate seems to decrease in amount as samples are obtained higher in the series. Still there are appreciable quantities present. If this material were spread out and weathered into soil without any loss or gain in soluble material, the soil would be extremely rich in salts soluble in water. Considering the average amount present, each acre would contain 108,502 pounds or over 54 tons of material soluble in water per two million pounds of soil, which is roughly the weight of the plowed surface of soil to a depth of 6⅔ inches. There also would be 892 pounds of sodium nitrate in this layer of soil. It should be remembered that the usual application of sodium nitrate as a fertilizer is about 200 pounds

TABLE III

NITRIC NITROGEN CONTENT OF THE COUNTRY ROCK EAST OF ST. GEORGE, UTAH

Field No.	Description and location of samples	Total soluble salts p. p. m.	Nitric Nitrogen	
			Parts per million	Per cent of total salts
336	Dark shale on opposite side of hill from No. 335	6,560	468.00	7.13
341	Clay from same locality as No. 340	70,080	64.40	0.090
338	Anhydrate gypsum just below No. 337	44,600	46.80	0.105
343	Shale north of bridge over Virgin River	37,600	35.10	0.093
344	Crest over shale near No. 343	109,320	35.10	0.032
337	Gypsum in valley on road to Hurricane	44,800	30.40	0.068
339	Shale interstratified with gypsum	46,800	22.40	0.047
335	Shale east of Washington	5,560	17.55	0.316
340	Cherty limestone east of Washington	46,440	11.70	0.025
342	Alkali just under sandstone	130,800	11.70	0.009
	Average	54,256	74.31	0.79

per acre. Is it any wonder that the application of irrigation water to such soil gives rise to the concentration of nitrates in spots which are rich in nitrates? To emphasize further the significance of the data, apply the same method of reasoning to sample No. 336. The plowed surface of such a soil would contain 5,816 pounds or nearly three tons of sodium nitrate per acre. With the proper control of the irrigation water this material is of great economic importance in the production of crops.

IV. The Country Rock of North Arizona

Nine samples of shale, clay, gypsum and alkali were collected in northwestern Arizona. The results of analysis are recorded in Table IV. The largest amount of water-soluble salts and nitrate are found in samples No. 350 which represents the clay crust at the base of the shale cliffs.

These samples are not so rich in nitrates as those in southern Utah, although again much more nitrate is present than is found in our best cultivated soils.

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V. The Country Rock at Mt. Carmel

In Table V are recorded the results of analysis of material collected near Mt. Carmel, Utah, which is located about 100 miles from St. George

TABLE IV

NITRIC NITROGEN CONTENT OF THE COUNTRY ROCK IN NORTHERN ARIZONA

Field No.	Description and location of samples	Total soluble salts p. p. m.	Nitric Nitrogen	
			Parts per million	Per cent of total solids
350	Clay crust at base of shale cliffs west of Fredonia, Ariz.	141,240	70.2	0.050
345	White gypsum east of Workman's spring	47,400	40.9	0.086
346	Red shale and gypsum near No. 375	46,240	40.9	0.089
352	Red shale	44,640	23.4	0.052
353	Red sandstone	1,520	23.4	1.540
347	Hard white material near Pipe Springs	44,920	11.7	0.026
348	Ash near Fredonia	48,600	11.7	0.024
349	Brownish red shale 3 miles west of Fredinia	45,920	11.7	0.026
351	Shale overlying the red shale	46,240	11.7	0.025
	Average	51,858	27.3	0.212

up the valley of the Rio Virgin. In fact the river here has not cut through the Triassic deposits, but is now making a valley through the Cretaceous materials similar to those near Grand Junction, Colorado.

TABLE V

NITRIC NITROGEN CONTENT OF THE COUNTRY ROCK NEAR MT. CARMEL, UTAH

Field No.	Description and location of samples	Total soluble salts p. p. m.	Nitric Nitrogen	
			Parts per million	Per cent of total solids
364	Sandy shale	118,120	152.0	0.128
366	Wet sticky clay	35,800	146.0	0.408
360	Red shale at base of hill	3,720	123.0	3.310
361	Red clay from base of hill	4,920	87.8	1.780
359	Near Mt. Carmel	2,720	76.0	2.790
357	Impure gypsum near Mt. Carmel	40,520	76.0	0.188
365	Brown shale	69,360	64.3	0.093
367	Disintegrated shale	38,320	58.5	0.153
362	Black coal shale	16,160	46.8	2.290
363	Argillaceous shale	9,720	29.3	0.301
358	Red shale at Mt. Carmel	3,560	23.4	0.065
355	Red sandstone higher up than No. 354	1,840	23.4	1.220
354	Red sandstone in Kanab canyon	1,920	23.4	1.220
356	Red grit at Three Lakes, Kanab canyon	1,600	17.7	1.100
	Average	17,734	67.7	1.070

An examination of the data presented shows that the original rock is rich in salts including the nitrates, although not so rich as some of the other deposits previously studied belonging to this same series.

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VI. The Country Rock at Cedar City

The rock material collected near Cedar City, Utah, probably belongs to the Tertiary deposits. The results obtained from the analysis of these samples are recorded in Table VI.

TABLE VI

NITRIC NITROGEN CONTENT OF THE COUNTRY ROCK NEAR CEDAR CITY, UTAH

Field No.	Description and location of samples	Total soluble salts p. p. m.	Nitric Nitrogen	
			Parts per million	Per cent of total salts
376	Gypsum from old tunnel near No. 375	47,640	93.5	0.196
368	Drab shale on "State" road to Mt. Carmel	9,920	58.5	0.590
369	Gypsiferous shale northeast of Cedar	48,160	41.0	0.085
377	Fine red ashy dirt near No. 376	50,440	29.3	0.058
378	Red clay from salt mines east of Cedar	21,640	23.4	0.108
370	Gypsiferous shale east of No. 369	46,200	11.7	0.025
371	Yellow ledge, disintegrated shale	41,600	11.7	0.028
372	Crust from top of shale	42,080	11.7	0.028
373	Shale in the ledge	42,960	11.7	0.027
374	Shale near intake to power system for Cedar	2,160	11.7	0.540
375	Red shale with gypsum underneath No. 374	43,800	11.7	0.027
	Average	36,055	28.7	0.160

The rock here is not quite so rich in nitrate as the Cretaceous or Triassic collected in the more southern part of the state, but still there is an appreciable quantity of nitrate. The alkali is more uniformly high in all the samples collected in this Tertiary material. An entirely similar condition was observed in eastern Utah. The Tertiary deposits were

TABLE VII

SUMMARY

District	Total soluble salts p. p. m.	Nitric Nitrogen	
		Parts per million	Per cent of total salts
North of St. George, Utah	29,989	81.4	0.71
West of St. George, Utah	48,962	156.0	4.22
East of St. George, Utah	54,256	74.31	0.79
Northern Arizona	51,858	27.3	0.212
Vicinity of Mt. Carmel, Utah	17,734	67.7	1.07
Vicinity of Cedar City, Utah	36,055	28.7	0.16

richer in alkali and poorer in nitrate than the corresponding Cretaceous deposits. There is a far greater extent of Tertiary material exposed, however, and a greater area of soil derived from this kind of rock. Both the alkali and nitrate are more uniform in these deposits.

The average data for these various districts are brought together in Table VII. With two exceptions, less than one per cent of the total solu-

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ble material is nitrate. These exceptions are in the upper part of upper Cretaceous at Mt. Carmel, and the Triassic, low in the series, west of St. George.

In the Cretaceous our results seem to indicate a decrease in nitrate with a decrease in the other water-soluble salts, while in the Triassic exactly the opposite condition is found, i. e., an increase in the nitrate content. All sections investigated are characterized by a high total water-soluble salt and nitrate content.

VII. Relationship of Nitrate Content Rock and Cultivated Soils

It should be remembered that it is only when the nitrates of the country rock are concentrated by the movement of the underground water over large areas that accumulations or spots occur in the cultivated soils. The abundance of nitrate occuring in the undecomposed shale adjacent to and underneath the cultivated soil is large enough to supply fully all the nitrate and other alkali salts present in these nitre spots.

The data presented here and in the previous publications are conclusive regarding the source of the nitrate accumulations in certain western soils. It is clearly demonstrated that the country rock adjacent to, underneath, and out of which the agricultural lands have been formed is abundantly supplied with the alkali salts and unusually large amounts of nitrates. Wherever and whenever the proper moisture conditions occur there is an accumulation of the alkali and nitrate in such large quantities as to render crop growth impossible. This condition occurs both in uncultivated and cultivated soils.

Such accumulations have been observed by us in many cultivated and virgin areas of Colorado, Utah, Wyoming and Alberta, Canada. In Colorado and Utah, the accumulations of alkali salts and nitrates have become so marked as to cause the death of many orchard trees. The source of the nitrate is directly traceable to the shale or sandstone, contributing to the soil or subsoil formation. The character of the exposed material may be noted from the accompanying photograph, Plate I, figure 2, taken of the shale hills northeast of Grand Junction, Colorado. The nature of the weathering and character of native vegetation may also be noted. The tops of the outlying clay hills are barren and close examination shows that under the clay crust there is an ash-like condition, previously noted, which is rich in alkali salts and nitrate.

This figure represents the upper part of the valley of the Grand River near the foothills. The agricultural land lies near the river. In these lands the river wash has filled in the depressions between the hills. The irrigating canals are higher up and in many places cut these clay hills near the upper part. The shale strata being nearly horizontal, the irrigating water penetrates the shale and follows the layers of shale down-

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ward to a point where the shale outcrops. The water rising to the surface produced a bog, spring or characteristic "soap hole" and on the evaporation of the water the salts including the nitrate are deposited, producing a nitre spot. In many of the cultivated lands in Colorado and Utah the lower point is covered with river wash two or three feet deep, but the water, under more or less pressure carrying in solution the nitrate and alkali, comes to the surface giving rise to the phenomenon noted. The greater the amount of water used, of course, the greater the deposition of salts. Consequently, it was observed in some sections that during the extremely dry years of 1910 and 1911 the trouble was more pronounced than usual. During a normal or wet season there is not so much water *maintained in the irrigating canals* and consequently not so much passes through the clay strata and the trouble is not so pronounced. Since these depressions are covered with river wash, one can very easily be led astray into assuming that the land is level and uniform. The surface may be level and uniform but the trouble arises from the *shale strata underneath.*

In many areas the shale and sandstone are not exposed, still the trouble from alkali and nitrate accumulations is manifest. Such a condition may be observed in the Great Plains area, and is well illustrated by conditions in Central Alberta, Canada. In Alberta the surface soil is of a loess character. Recently a new irrigation system has been installed and in some instances trouble has occurred from alkali accumulations arising apparently on the highest part of a given farm. The alkali incrustations have the characteristic brownish color due to the presence of the nitrates. What is the origin of this trouble since the shale and sandstone is not exposed? Close observation by digging or examination of other natural excavations, such as river banks, will show that the Cretaceous shale is present in this entire area and is merely covered over by deposits of glacial soil material to varying shallow depths. Where the alkali and nitre indications arise the undecomposed shales may be often found at from 3 to 6 feet. This shale is of the same geological age as that in Utah and Colorado, and is rich in soluble salts.

In Alberta where the trouble is experienced, the irrigating canals, as in Utah and Colorado, have cut the alkali bearing strata higher up. The water follows, often for two or three miles, the almost horizontal strata until it outcrops at the surface on some gentle slope in the form of an alkali bog, or as it is locally called "soap hole," which is at first very small in size but gradually increases until large areas of land are ruined. Whenever the alkali and nitre spots are evident, close examination will reveal the presence of the alkali or nitrate bearing shale, sandstone or other rock.

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VIII. The Relationship of the Alkali to the Nitrate Accumulations

The nitrates are not uniformily distributed through the affected areas. Certain spots may be rich in nitrate while others contain comparatively little nitrate. Exactly the same condition may be observed with the chlorides or sulphates. Some spots may be largely composed of chlorides, while others may consist almost entirely of sulphates. This condition was observed in the study of the alkali content of the country rock. Certain samples of country rock from some areas were rich in the nitrate, while others from different areas were poor in nitrate. But in any given area or spot of cultivated soil the chlorine and nitric nitrogen increase or decrease in the same proportion, thus indicating a common origin. There is no reason to assume, however, a more uniform distribution of the nitrate than of the chloride or sulphate in the conception that the nitrates are derived from the preëxisting nitrates in the country rock.

The nitrates are not independent of the other alkali salts. Cases may be observed where the alkali is largely the sulphate or chloride, and, if so, the surface indications are white in nature. No case has been observed or reported where the nitrate accumulations occur independent of either the chlorides or sulphates. Consider, for example, a typical case cited by Dr. Headden to which attention has already been called by us (11). In 1907, a typical nitre spot (case 8) contained in the surface foot traces of nitric nitrogen, 20,217 pounds of chlorine and 98,000 pounds of total salts. Four years later new determinations were made at the same spot with the following results: nitric nitrogen 621 pounds, chlorine 257,000 pounds, total salts 727,040 pounds. Is it not absurd to claim that nitrate is free from alkali when 1/3 ton of nitrogen is accompanied by 128 tons of chlorine and 313 tons of total salts? And the increase of 1/3 ton of nitric nitrogen has been accompanied by an increase of 118 tons of chlorine and 313 tons of other salts. That is, in this characteristic nitre spot in cultivated soil the nitric nitrogen is only 0.08 per cent of the total salts present. An examination of the data presented by us will show that there are few cases where the proportion of nitric nitrogen to total salts in the country rock is so small as this. Furthermore, we can hardly conceive of the addition of 313 tons of the chlorides and sulphates of sodium, potassium or magnesium to even our best agricultural soils and expect to obtain any growth of crops at all. The nitre spots are not independent of these other salts.

Entirely similar conditions have been noticed by us in our examination of the country rock. Small amounts of nitrate have been accompanied by large amounts of other salts in the rock itself *with local concentrations of nitrate accompanied by the other salts in the ash and alkali*

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crust at the surface wherever moisture conditions are such as to cause a large evaporation of the alkali and nitrate bearing water.

And certainly there is no question that the alkali is due in a large measure to the marine deposits in the shale and deposited in the old epicontinental seas of upper Triassic, Cretaceous, and Tertiary times. The shales and sandstones are abundantly supplied with those salts. The Triassic below St. George, Utah, contains over 50 tons of salts per acre to a depth of 6⅔ inches. The Tertiary shales in the Uinta Basin are so rich in salts that costly attempts to build irrigating canals through these shales have been a failure, due to the dissolving and disappearing of the canal banks, being carried away in solution by the irrigating water. The nitrate accumulations are not independent of the alkali salts but intimately connected with them.

IX. The Relationship of the Non-Symbiotic Bacteria to the Nitrate Accumulations

As the nitre spots slowly develop by the concentration of the alkali salts which also include the *nitrates* and *phosphates* of calcium, magnesium, sodium and *potassium,* ideal conditions are temporarily produced for plant nutrition. For two or three years before the soil is rendered non-productive phenomenal growth of crops is obtained due to the presence of all plant-food in the highly available condition. Conditions for the growth of the bacteria must also be temporarily more favorable. It is not surprising, therefore, that the non-symbiotic bacteria, especially the azotobacter, have been observed in these spots and undue significance placed on their presence. As the nitre spots develop, however, the concentration of alkali and nitrates becomes too great for the growth of either the higher or lower plants.

The fixation of nitrogen by the non-symbiotic bacteria in the ordinary dry-land soil in Utah, which is free from alkali and nitrate accumulations, is far greater than in the nitre areas, and yet we find no nitrate accumulations in such soils. Thus Sackett (7, p. 28) reports a maximum fixation of 15.8 mg. of nitrogen, per 100 gm. of soil from the nitre areas, *while Greaves* (2) *reports a maximum of* 51.8 *mg. of nitrogen fixed in the soils of Utah which are free from the nitrate accumulations.* The average amount fixed in the 32 samples collected by Sackett in Colorado from the nitre areas was 7.4 mg., *while Greaves found an average amount of* 12.2 *mg. in the* 31 *samples of dry-farm, alkali-free soil in Utah.*

These same dry-farm soils, which show a far greater fixation of nitrogen than the nitre soil, have been shown (8) to contain only normal amounts of nitrates. The amount in no case exceeds 6 parts per million, yet these soils have been cultivated for a period of over fifty years. The non-symbiotic bacteria, which are *active* and more *abundant* in the adja-

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cent virgin soil than in the nitre soils, have had unlimited time during the past ages to produce the nitrate, yet the average nitrate content does not exceed 1.04 parts per million as an average of 22 separate determinations from soil of a wide area in the Cache Valley. *These dry-farm soils are also alkali-free.*

Finally, it must be remembered that the nitre spots are always characterized by a brown color. The nitrates themselves are colorless. The color is due to colored organic material. Bacteria are not necessary even for the production of this color.

Characteristic nitre spots have been produced by us at will in the laboratory in the following manner: 300 gm. of a rich greenhouse soil were weighed out into an evaporating dish which was then filled with a 10 per cent solution of sodium nitrate and allowed to evaporate slowly by the sun's rays without disturbing the dish or soil. When the soil became dry all of the characteristic indications of the nitre spots were present. The surface soil was highly colored, brownish in appearance. A thin crust was present underneath which was the characteristic mealy condition noted in the field. The artificial nitre spots were typical and they could be produced in this soil by adding the same amount of nitrate in the solid form and irrigating the soil with a saturated solution of mercuric chloride, or a 5 per cent solution of carbolic acid. On the crystals of mercuric chloride, which were finally deposited on the surface of the soil, was also deposited the rich brown color or "pigment" characteristic of the nitre spots. The soil itself, however, was harder than in the absence of the antiseptic. In a check experiment, evaporation of an equivalent amount of water from the same amount of soil failed to produce the color or other characteristic indications of the nitre spots. Surely this definitely eliminates the bacteria as the cause of the production of color as definitely as they have been eliminated as the cause of the production of the nitrates. The non-symbiotic bacteria are not essential to the production of the color or the nitre spots. The bacteria themselves are only incidental and under the given conditions are of no economic significance whatever.

The color is produced by the solvent and decomposing action of the nitrate, especially sodium and potassium nitrate upon the organic matter of the soil. In all soils or manure, there occur more or less considerable amounts of colored organic compounds, the solubility of which can be increased by various kinds of treatment. Thus, in the official humus determination it is necessary to treat the soil with an acid to decompose the calcium or magnesium salts of the organic acids present, after which they are readily soluble in the ammonia solution. The calcium or magnesium salts are insoluble, while the ammonium salt is soluble and *highly colored.* Likewise, in the soil of the nitre spots the calcium or magnesium salts of the colored organic acids are insoluble but are converted

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into soluble compounds by double decomposition when sodium or potassium nitrate is present as already described (14).

X. Nitre Soils Not Limited to a Single Geological or Geographical Area

The nitre soils have been observed in widely separated portions of Utah, Colorado and Wyoming. They have been observed in cultivated and uncultivated soil derived from the shales, sandstones, limestones and marls of the Cretaceous, Tertiary, Juriassic, Triassic and Permian. The conditions are more pronounced in the soils derived from the Cretaceous and Tertiary. The nitre soils have been observed also in the Great Plains Area such as Central Alberta, Canada. While the conditions are more pronounced in arid soils, similar conditions have been observed in humid soils.

An interesting case of a characteristic "nitre spot" occurring in a humid climate is reported by Cameron (1, p. 36), although not recognized by him as such, possibly because his report was made prior to the discovery of the importance of the problem in the Arid West. This particular spot occurred on the Maryland Experiment Farm at College Park and was first reported by Professor Whitney. A sample of this material was secured and on analysis gave the following results:

$CaSO_4$	$CaCl_2$	$Ca(NO_3)$	$Mg(NO_3)_2$	$NaNO_3$	KNO_3
1.86	7.55	51.20	20.68	12.47	6.24

That is, over 50 per cent of this material is calcium nitrate, while over 90 per cent of the soluble parts is in the form of nitrates. The total soluble material in the "crust" was 1.83 per cent. This spot had the characteristics of the nitrate spots occurring in the Arid West including the hard crust on the surface and the "dark brown color." The characteristics are all present: (1) a high nitrate content, (2) a hard crust, (3) presence of other soluble salts including chlorides and sulphates, (4) dark brown color or "pigment." No mention is made of the mealy condition underneath the crust, but since this is due to the presence of soluble salts, undoubtedly closer examination of this spot would have revealed its presence. An examination of the country rock underneath or adjacent to this soil would certainly be interesting.

XI. Utilization and Reclamation of the Nitre Soils

The amount of the nitrate in a widely disseminated condition in the shales and sandstones of the area is enormous and if its distribution be properly controlled by the intelligent use of the irrigation water so as to prevent undue accumulations important economic use may be made of the nitrate present. When, however, the accumulations become so excessive as to prevent plant growth resource must be made to artificial drainage.

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But one of the very discouraging features of this trouble is the fact that the ordinary type of drainage is inadequate to obviate the condition. Often drains established in the usual manner will not be flowing, yet the condition complained of is very pronounced on parts of the farm. This is due to the close, impervious structure of the soil which does not readily permit the lateral movement of water. Special drainage adapted to this condition by tapping the source of water which produces the bog or "soap hole" with a drain, will become effective. The condition also will be much benefited by cementing the irrigating canals at the point of contact with the alkaline shale ridges, thus preventing the direct entrance of the irrigating water.

One must not be led astray regarding the necessity of drainage from a study of the deep underground water supply. Misleading information may be obtained from a study of the wells used for domestic water supply. Our investigations have clearly demonstrated that some members of the geological series of shales and sandstones contain greater quantities of salts than others, and that some members are largely free from salts. The farmer in digging a well seeks good drinking water and usually finds it by driving in the river wash or through the alkali and nitrate bearing strata. This is well illustrated at Lyman, Wyoming. The shallow wells are all so charged with salts as to render them unfit for drinking purposes. Deeper wells which penetrate through the alkali-bearing strata into gravel and sand underneath furnish good drinking water.

Acknowledgment

The authors wish to acknowledge their indebtedness to Mr. C. T. Hirst and Mr. Percy Shelly who made the analysis for nitrate and total alkali content of the country rock.

Conclusions

1. Certain soils in western arid America contain spots which are rich in nitric nitrogen and contain in some instances several hundred times as much nitric nitrogen as occurs in normal cultivated productive irrigated soil.

2. These spots are locally known as "nitre spots."

3. Three theories have been presented regarding the origin of these accumulations: (a) Hilgard's conception was that the accumulations were due to the more rapid nitrification of the organic matter of the soil in arid climates. (b) Headden maintains that they are due to the fixation of the atmospheric nitrogen by the non-symbiotic bacteria. (c) Stewart and Peterson believe that they are the direct result of the leaching of the nitrates out of the preëxisting deposits in the country rock and locally concentrated by seepage.

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4. The country rock adjacent to the affected areas and which has contributed to the soil formation is rich in nitrate and contains such an abundance of this material as fully to account for the accumulations noted.

5. The nitrate is transported from the original rock by means of seepage water. It is transported more rapidly where the water escapes from leaky irrigating canals cutting shale strata, thus allowing the seepage water to follow the shale strata to the point of outcrop. Nitre spots are produced where such seepage water rises and evaporates.

6. The nitrate deposits are not limited to the country rock of a definite geological series. They have been found in large quantities in the Cretaceous, Tertiary and Triassic with smaller quantities in the Jurassic.

7. The nitrate accumulations occur in the uncultivated soil wherever the water conditions are such as to cause the concentration of the nitrates in any given area.

8. The accumulations in the virgin soil possess all the characteristics of the nitre spots in cultivated soil, i. e., (a) a high nitrate content, (b) the presence of excessive quantities of other soluble salts, (c) brownish color, (d) thin crust over surface, (e) mealy or ash-like condition under the hard crust, and (f) a moist, sticky, glistening soil beneath the ash material.

9. Characteristic nitre spots may be produced artificially in the laboratory with a rich greenhouse soil and an excess of sodium nitrate. They are produced equally as well in soil rendered sterile by treatment with a saturated solution of mercuric chloride or 5 per cent solution of carbolic acid.

10. The non-symbiotic bacteria are not essential to the production of the nitrates or of the color found in the nitre spots. Their presence in these nitre soils is only incidental.

11. The nitrates of the nitre spots of the cultivated soils are derived from the preëxisting accumulations occurring in the adjacent country rock and transported by the movement of underground water brought to the surface by exposure of the rock outcrop and there deposited by the evaporation of the water.

12. The brown color of the nitre spots is due to the solvent and decomposing action of the nitrates on the old organic compounds of the soil.

13. The source of this old organic matter, like the nitrate, is in the shale and sandstone of the area which is coal and oil bearing.

14. The nitrate accumulations occurring in the country rock, like those noted in the soil, are always accompanied by excessive accumulations of other water-soluble salts.

15. The alkali salts in the affected area are derived from the preëxisting salts occurring in the country rock.

16. These nitre spots are not limited to any single geological horizon or geographical area.

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Literature Cited

(1) Cameron, T. K.
1901. Soil solutions: their nature and functions, and the classification of alkali lands. U. S. Dept. Agr. Bur. Soils Bul. 17, 39 p.

(2) Greaves, J. E.
1914. A study of the bacterial activities of virgin and cultivated soils. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 41, p. 444.

(3) Headden, W. P.
1910. The fixation of nitrogen in some Colorado soils. Colo. Agr. Exp. Sta. Bul. 155, 48 p., 8 pl.

(4) Headden, W. P.
1911. The fixation of nitrogen in some Colorado soils: a further study. Colo. Agr. Exp. Sta. Bul. 178, 96 p., 6 pl.

(5) Headden, W. P.
1913. The fixation of nitrogen in Colorado soils: the distribution of the nitrates and their relation to the alkalis. Colo. Agr. Exp. Sta. Bul. 186, 47 p.

(6) Hilgard, E. W.
1906. Soils. 593 p., 89 fig. New York.

(7) Sackett, W. G.
1911. Bacteriological studies of the fixation of nitrogen in certain Colorado soils. Colo. Agr. Exp. Sta. Bul. 179, 42 p., 2 pl., 5 fig.

(8) Stewart, R.
1910. The nitrogen and humus problem in dry-land farming. Utah Agr. Exp. Sta. Bul. 109, 16 p.

(9) Stewart, R.
1913. The intensity of nitrification in arid soils. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 36, p. 477-490.

(10) Stewart, R., and Greaves, J. E.
1909. A study of the production and movement of nitric nitrogen in an irrigated soil. Utah Agr. Exp. Sta. Bul. 106, p. 65-96.

(11) Stewart, R., and Greaves, J. E.
1911. The movement of nitric nitrogen in soil and its relation to nitrogen fixation. Utah Agr. Exp. Sta. Bul. 114, p. 179-194.

(12) Stewart, R., and Greaves, J. E.
1912. The production and movement of nitric nitrogen in soils. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 34, p. 115-147, 1 fig.

(13) Stewart, R., and Peterson, W.
1914. The nitric nitrogen content in the country rock. Utah Agr. Exp. Sta. Bul. 134, p. 419-465.

(14) Stewart, R., and Peterson, W.
1915. The origin of the "nitre spots" in certain western soils. *In* Jour. Amer. Soc. Agron., v. 6, no. 6, p. 241-248.

(15) Warrington, R.
1892. Investigations at Rothamsted Experiment Station: Nitrification of soils and manures. *In* U. S. Dept. Agr. Off. Exp. Sta. Bul. 8, p. 77-113, pl. 9-11.

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PLATE I

Fig. 1. Photograph of typical clay hill north of St. George, Utah, from which samples of shale were taken.

Fig. 2. Photograph taken in the shale hills northeast of Grand Junction, Colorado, showing the character of the exposed material, the nature of the weathering and the character of the native vegetation.

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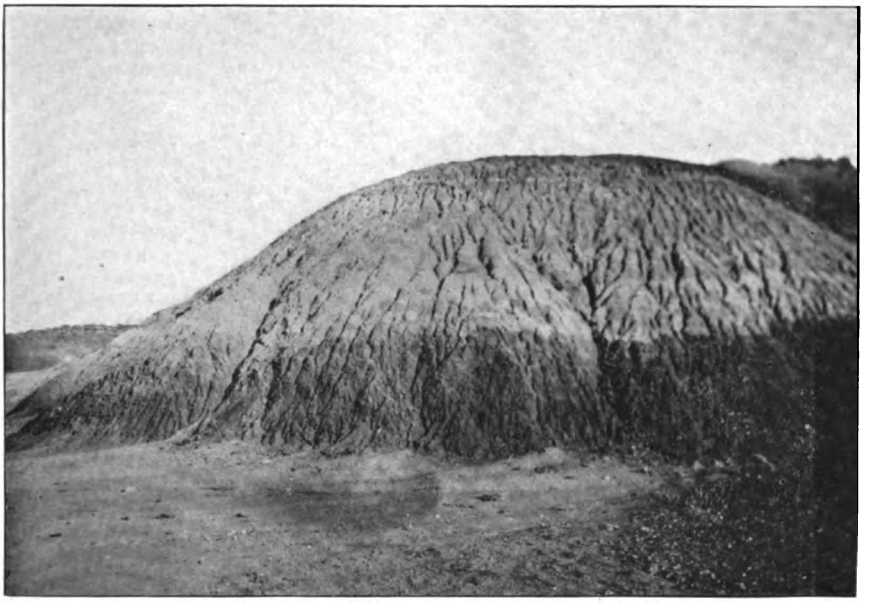

Fig. 1

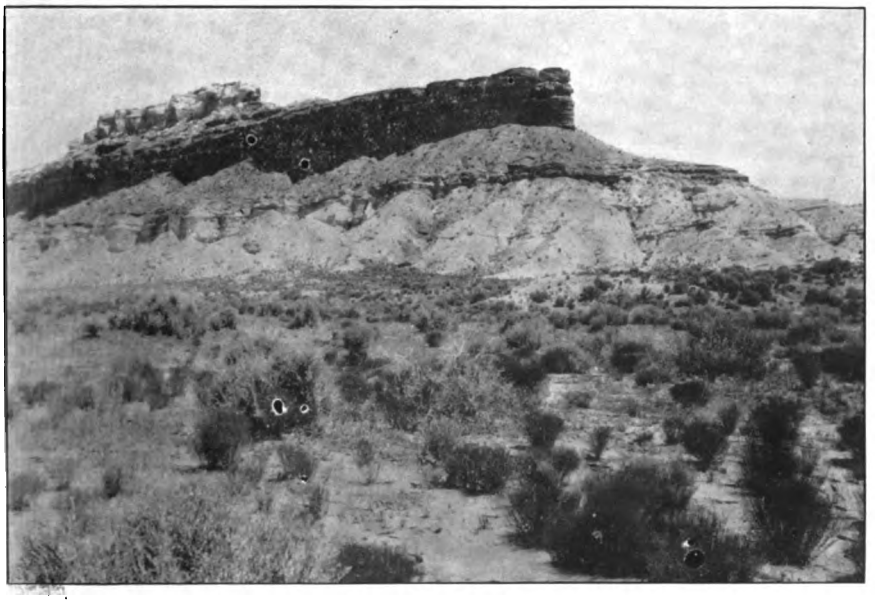

Fig. 2

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PROTOZOA, AS AFFECTING BATERIAL ACTIVITIES IN THE SOIL[1]

By

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INTRODUCTION

The theory proposed by Russell and Hutchinson (7, 8) that the protozoa act as a factor detrimental to soil bacteria and, for that reason, to soil fertility, has resulted in a great deal of work on the life of protozoa in the soil in general, and particularly on their behavior toward partial sterilization. The latter is supposed to destroy the protozoa, allowing the bacteria to multiply rapidly and liberate a larger amount of plant-food. Some of the data brought to light by these investigations goes to support this theory, while much of the work, particularly that of American investigators, does not find any basis for its justification.

HISTORICAL

Without taking up the full bibliography on the subject, since it can be found in most of the recent publications, attention will be called only to a few of the recent investigations which have a direct bearing upon the work to be presented. Russell and Hutchinson (7, 8) have shown that there are organisms in the soil which act detrimentally upon bacterial numbers and activities; the number of bacteria found in the soil at any time is not a simple function of the temperature, moisture content or other conditions of the soil, but depends on the difference in activity of the bacteria and the detrimental organisms. After the detrimental organisms are killed by antiseptics or heating the soil to 55° to 60° C. there is a rise in bacterial numbers and ammonia production in the soil; the detrimental organisms have been identified with the active protozoa in the soil.

This influence of antiseptics upon soil and the microörganic activities in it has been subjected to a great deal of study. Several investigators claim that the antiseptics act as a direct stimulant upon the bacterial flora of the soil. Hiltner and Störmer (4) believed that the bacterial flora of the soil consists of harmful and beneficial organisms; the antiseptics suppress the activities of the harmful bacteria, thus allowing the beneficial ones to multiply rapidly and liberate a greater amount of plant-food.

[1] Received for publication July 29, 1916.

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The action of heat upon soil is explained by some investigators to be due to the destruction of toxins in the soil, although the presence of such substances has not been as yet definitely established. Greig-Smith (3) did not find any limitation in the number of soil bacteria upon adding suspensions of protozoa. Comparatively large amounts of volatile antiseptics when added to the soil did not destroy even the large ciliates. The effect of heat was of a different character upon the growth of bacteria. He concluded that the toxins and nutrients of the soil are alone concerned with the changes that occur when soils are heated or treated with volatile antiseptics; the protozoa have little or no action in limiting the numbers of soil bacteria.

Working with "sewage-sick" soils, Russell and Golding (6) have shown that they can be improved by partial sterilization: antiseptics and heat will put the detrimental factor out of action; the detrimental factor was similar to the one found by Russell and Hutchinson (7, 8) in ordinary soils, but was more pronounced. Similar results have been obtained by Russell and Petherbridge (9) in their study of "greenhouse-sick" soils. Russell and Buddin (5), studying the effect of antiseptics upon soil, found that toluene caused first a decrease and then an increase and also a change in type in the microörganic population of the soil, the suspension of nitrification and a marked increase in ammonia production. Gainey (2) reported that small quantities of CS_2, toluol and chloroform exerted a stimulating effect upon the bacterial numbers, but had no appreciable effect upon the number of types of protozoa present. Cunningham (1), adding cultures of protozoa to bacteria, concluded that soil protozoa exercise a decided limiting effect on the numbers of bacteria and their activities in the soil.

The author (12) has shown previously that the presence of protozoa acts detrimentally upon bacterial numbers in the soil, but has no appreciable influence upon ammonification by bacteria. This detrimental effect of protozoa upon the bacterial numbers and their non-detrimental effect upon ammonification in the soil was explained by one of the following assumptions: (1) if the protozoa destroy bacteria, they destroy non-ammonifying organisms; (2) the protozoa themselves may take part in the process of ammonification; or (3) the disintegration of the bacterial cells results in decomposition products which might be responsible for high ammonia production.

Sherman (10) found by using a synthetic bacterial soil flora, free from protozoa, that the soils containing protozoa gave lower bacterial counts. He has shown further that volatile antiseptics may destroy some protozoa, but not all of them, leaving ciliates, flagellates and amoebae. The maximum number of bacteria was not found while the protozoa were depressed by the action of antiseptics, but the development both of bac-

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teria and of protozoa subsequent to treatment with volatile antiseptics ran parallel. Bacterial flora in soils containing protozoa and free of protozoa behaved in exactly the same way when exposed to different conditions of temperature and moisture content.

It is seen from the short historical review presented that the question of influence of protozoa upon bacterial numbers and activities in the soil, as well as of the actual conditions created in the soil by the action of antiseptics, which stimulate bacterial activities, is still undecided. One of the errors made by several investigators is the comparison of results obtained from entirely different soils. The soils of this country are different in their content of organic matter and perhaps of protozoa, from the "sick" soils of England. Dr. Löhnis has suggested to the author that there might be two classes of protozoa in the soil: useful and harmful. In ordinary cultivated, well aerated soils with a low content of organic matter, the useful protozoa may predominate. While, when soil is kept under certain conditions, such as high temperature, high moisture and high content of organic matter for a long time, harmful protozoa may develop, and this condition may lead to soil "sickness," as in the case of the "greenhouse" and "sewage-sick" soils studied by Russell and his associates. The problem was then to try to produce "sick" soils, the history of which should be known, as well as the activity of protozoa in those soils. This paper is limited to the study of the interaction between protozoa and bacteria in the soil, without going into details concerning the occurrence and numbers of protozoa in the soil.

Experimental

Methods of study. A clay soil classed by the Bureau of Soils as Penn loam and a light Sassafras loam were used for this work. The soils were divided into 3 portions: to one was added 20 per cent of fresh stable manure, and to another 20 per cent of finely cut green alfalfa; the third portion was left untreated. The soils were distributed, in 100-gm. portions, in tumblers, and moisture added equivalent to 70 and 100 per cent of the water-holding capacity of the soils. The tumblers containing the soils thus differently treated were then incubated at 3 different temperatures: one set at 30° C. in an electric incubator; the other set in the laboratory, the temperature of which ranged from 18° to 22° C.; and the third set was placed out-of-doors and kept there from October 19, 1915, when the experiment was started, until the beginning of July, 1916, when it was completed; a few tumblers were lost during that period, due either to the action of the frost outside, or to breakage.

The sets of tumblers representing the two soils were treated differently. Out of the tumblers containing the Sassafras soil representative samples, for the determination of bacterial numbers and their ammonify-

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ing efficiency, were taken at the end of 37, 156 and 230 days after the experiment was started. At the end of the experiment (after a period of 230 days) the soils were divided into 3 portions: one set was left as a check; the second set was treated in Erlenmeyer flasks at 65° C. for 5 hours; the third set was treated with 4 per cent toluene for 48 hours, then spread out, and the toluene was allowed to evaporate for 48 hours. After the bacterial numbers were determined in the three sets differently treated, the ammonifying efficiency of each portion was also determined.

The set containing the Penn loam soil was kept untouched until the end of the experiment (230 days). The 100 gm. of soil from each tumbler was then divided into 3 portions; one of these portions also was treated with toluene, one with heat, and one portion was left untreated, in a manner similar to the Sassafras soil portions. However, the bacterial numbers were determined in the toluene treated soils at the end of 48 hours without allowing the antiseptic to evaporate, since, as was found in the case of the Sassafras soils, the bacteria multiplied rapidly during the 48 hours of evaporation. The proper amount of moisture was then added to the portions thus treated and allowed to incubate at 22° C. for 30 days, after which period bacterial counts were made again and the amount of ammonia formed in each portion determined by distilling with MgO.

The bacterial numbers were determined by plating out a 200,000 dilution on modified albumen agar (11). The ammonifying efficiency of the bacteria was determined by sterilizing 100 gm. of Sassafras soil to which 155 mg. of N. in the form of dried blood and proper moisture content were added, then inoculating with 1-c.c. suspensions of the proper soil made by shaking for 5 minutes 10 gm. of the soil with 100 c.c. of sterile water. These were allowed to incubate for 12 or 28 days and the amount of ammonia determined by distilling with MgO. In the case of the Penn loam soils this method of procedure was not followed; only the amount of ammonia formed in the soil itself was determined.

This experiment was not planned to be an exhaustive one; it was intended originally to get an idea for certain lines of attack upon the probable activities of protozoa in the soil. For that reason such small portions of soil have been used, since with the different treatments a large amount of space and time was required. The author merely wanted to learn whether one could get an idea about the possible relationship between the partial sterilization of the soil and the microörganic activities, by making the conditions favorable for the protozoa, and thus breeding them in the soil, so as to have side by side soils rich in protozoa and soils poor in protozoa; soils in which protozoa were known to lead an active life at all times, and soils in which no protozoa were ever found to be active; using the two types of soil made "sick" to a certain extent, as far as the protozoa go.

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The bacterial numbers and the ammonifying efficiency of the Sassafras soils differently treated are presented in Table I; the effect of partial sterilization by heat and toluene upon bacterial numbers and the ammonifying efficiency of the same soils is presented in Table II; and the results of the work with the Penn loam soils differently treated are given in Table III.

TABLE I

DEVELOPMENT OF BACTERIAL NUMBERS AND THEIR AMMONIFYING EFFICIENCY IN SASSAFRAS SOILS KEPT UNDER DIFFERENT CONDITIONS OF MANURE, MOISTURE AND TEMPERATURE

Soil Treatment			Protozoa, Active	Millions of Bacteria per gram of Soil			Ammonia Accumulated in mg. N. in 100 gm. of Soil plus Dried Blood		
Manure	Moisture %	Temperature ° C.		After 37 Days	After 156 Days	After 230 Days	After 37 Days[1]	After 156 Days	After 230 Days
Stable	70	out-of-doors	0	153.0	22.9	21.0	64.76	26.00	69.30
Stable	100	20	C.F.[2]	25.0	8.7	7.0	21.26	56.80	59.22
Stable	100	30	C.F.	3.0	5.8	1.2	31.95	22.30	73.58
Alfalfa	70	out-of-doors	0	50.6	45.6	41.2	70.64	93.60	64.89
Alfalfa	70	30	F.	13.8	9.2	11.2	12.21	21.50	73.71
Alfalfa	100	out-of-doars	C.F.	43.2	41.2	21.6	52.58	30.65	60.87
Alfalfa	100	20	C.F.	7.6	6.5	8.0	25.48	18.30	65.53
Alfalfa	100	30	C.F.	9.0	10.9	4.5	13.92	14.55	63.33
None	70	out-of-doors	0	2.4	7.6	4.9	58.40	61.30	72.45
None	70	20	0	2.8	6.9	3.0	68.58	58.60	68.04
None	70	30	0	1.0	1.9	1.1	69.77	16.90	77.83
None	100	out-of-doors	0	3.0	6.1	4.2	65.37	52.80	74.97
None	100	20	0	1.2	3.0	5.0	64.02	50.95	69.30
None	100	30	0	1.0	2.1	1.1	47.58	26.60	75.00
Average ...	.....	out-of-doors	.....	50.4	24.7	18.6	62.35	52.87	68.49
Average ...	.....	20	.....	9.2	6.3	5.8	44.84	46.16	65.52
Average ...	.....	30	.....	5.6	5.9	3.8	35.09	20.37	72.69
Average ...	70	.....	.....	37.3	15.7	13.7	57.39	46.30	71.04
Average ...	100	.....	.....	11.6	10.5	6.6	40.27	34.10	67.73

[1] After 37 and 156 days a 12-day incubation period was used for the determination of ammonifying efficiency; after 230 days a 28-day incubation period was used.

[2] C.—Ciliates; F.—Flagellates. Only active protozoa have been determined.

In Table I are shown the changes in bacterial numbers and their ammonifying efficiency in a Sassafras soil, containing varying quantities of manure and moisture, and kept under different temperatures. Protozoa were found in all soils, but were found to be active only in the soils to which 20 per cent of stable manure or green manure was added and at the higher moisture contents. This confirms the idea expressed before by the author (12) and also by Sherman (10) that the organic matter and high moisture content favor the activity of protozoa. The bacterial numbers were much higher in the manured soils than in the unmanured, which is self-understood, since the addition of available organic matter

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usually stimulates bacterial activity. But when the different periods are compared, an important difference will be found between the bacteria in the manured and in the unmanured soils; while the bacteria in the manured soils, with few exceptions, decreased steadily with the time of incubation, the bacterial numbers of the unmanured soils increased in every instance from 37 to 156 days, then slightly decreased to 230 days, always remaining above the first counts. This may be explained by the fact that in the soils containing a high percentage of organic matter in the form of stable manure or green manure, fermentation sets in readily and this stimulates bacterial activities; the final decomposition of the manure and perhaps the production of organic toxins may check the bacterial development after a certain length of time; this occurrence may not take place in the soils to which no manure has been added. There does not seem to be a great deal of difference between the ammonifying efficiency of the organisms from the manured and the unmanured soils. The different periods cannot be compared for the ammonifying efficiency of the microörganisms, since in the last case a longer incubation period was used.

As to the influence of the activity of protozoa upon the bacterial numbers, examination of the above table will show that there is probably a relationship between the two groups of microörganisms. The bacterial numbers were much higher in the absence of active protozoa (as far as could be shown by the present methods of study), and where the protozoa were found to be active there was a drop in bacterial numbers. This bears out again the contention of Russell and Hutchinson (7, 8) that the bacterial numbers are decreased in the presence of protozoa.

When the different temperatures of incubation are compared, the soils kept out-of-doors gave on the average, in all the three examinations, higher bacterial numbers and a higher ammonifying efficiency than those kept at 20° C., and the latter higher bacterial numbers and higher ammonifying efficiency (with one exception) than those kept at 30° C. The high temperature seems to have an injurious effect upon the bacteria; whether this is due to the lessening of the vitality of the organisms or to the more rapid decomposition of the organic matter, which sets free more organic toxins, cannot be decided as yet. The fact that the soils kept out-of-doors gave on the average higher bacterial counts in all instances, and stronger average ammonifying efficiency in all instances but one, may give support to the idea that bacteria are benefited by natural conditions and changes in temperature, since the soils froze and thawed many times during the winter. This cannot be explained by the fact that the low temperatures of the winter check the development of protozoa, and for that reason allow the bacteria to develop to larger numbers, as Russell and Hutchinson (8) maintain, since the soils kept outside had much higher bacterial numbers even before the freezing weather set in. Sher-

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man (10) also came to the conclusion that the low temperatures have no stimulating effect upon the bacterial cultures containing protozoa.

The high moisture content seems to act injuriously upon bacterial numbers and their ammonifying efficiency, since in most instances the optimum moisture gave higher bacterial counts and had a stronger efficiency for producing ammonia than the soils of the higher moisture content.

The results brought out in Table II will throw some light upon the possible influence of protozoa on the bacterial numbers and their ammonifying efficiency in the soil, also the rôle which partial sterilization plays in the killing of protozoa.

The heating of the soil at 65° C. for 5 hours killed all the protozoa, except in one case, where small flagellates were left. On the other hand, the treatment of the soil with 4 per cent toluene for 48 hours, which was then allowed to evaporate for 48 hours, killed only the ciliates and large flagellates, leaving the small flagellates; in one case small ciliates were found after the toluene treatment, while in another case the small flagellates were also killed. Thus, when one speaks of the killing of protozoa by partial sterilization, he should be careful to modify his statement as to what kind of protozoa he has in mind. The ciliates and flagellates are two distinct groups of organisms, and their rôle in the fertility of the soil also may be distinct. This confirms Sherman's (10) results, that partial antiseptics fail to exterminate entirely the protozoa in the soil.

Only the heating of the soil killed all the protozoa, and only in that case can one speak of a bacterial flora free from protozoa, while the toluene treatment freed the soil only from the larger types of protozoa. When these results are compared with the bacterial numbers of the soil and their ammonifying efficiency, the results will be found to be striking. The heating of the soil greatly reduced the bacterial numbers in all instances, the average being 1,700,000 per gm. of soil as compared with 8,300,000 for the check. The soils treated with toluene gave on the average a higher bacterial count than the check. This was due to the fact that the toluene-treated soils were allowed to evaporate for 48 hours and the bacterial numbers were allowed to increase, since the count was made only at the end of that period. When an equivalent amount of soil suspension (0.1 gm.) was added to 100 gm. of sterile soil containing 155 mg. N. in the form of dried blood and allowed to incubate for 28 days, the bacterial numbers and ammonia produced determined at the end of that period, the average numbers were found to be 33,000,000 for the soils inoculated with the untreated portions, 45,000,000 for the treated soil, and 25,800,000 for the toluened soil. It looks as if the presence of protozoa had some effect upon the bacterial numbers; the heated soils, free from protozoa, gave much higher bacterial numbers than those in which the protozoa were present. The results on the ammonia accumula-

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TABLE II

EFFECT OF HEAT AND TOLUENE ON THE BACTERIAL NUMBERS AND THEIR AMMONIFYING EFFICIENCY IN SASSAFRAS SOIL

Soil Conditions				Presence of Protozoa	Millions of Bacteria per gm. of Soil		Ammonia[1] in mg. N. Formed by 1 c.c. Suspension in 100 gm. of Soil plus Dried Blood
Manure	Moisture %	Temperature ° C.	Treatment		At the Beginning	After 28 Days	
Stable	70	out-of-doors	check	C.F.[2]	21.0	117 0	69.30
Stable ...	70	out-of-doors	heat	0	0.9	11.0	81.25
Stable	70	out-of-doors	toluene	F.	85.0	90.0	61.06
Stable	100	20	check	C.F.	7.0	30.8	59.22
Stable	100	20	heat	0	0.6	14.9	65.03
Stable	100	20	toluene	F.	10.4	49.6	63.63
Stable	100	30	check	C.F.	1.2	102.0	73.58
Stable	100	30	heat	0	0.7	13.7	71.74
Stable	100	30	toluene	F.	13.3	12.2	52.90
Alfalfa ...	70	out-of-doors	check	C.F.	41.2	13.1	64.89
Alfalfa ...	70	out-of-doors	heat	0	2.0	90.0	43.86
Alfalfa ...	70	out-of-doors	toluene	F.	94.0	99.7	59.49
Alfalfa ...	70	30	check	C.F.	11.2	9.9	73.71
Alfalfa ...	70	30	heat	0	7.6	6.9	29.24
Alfalfa ...	70	30	toluene	F.	10.0	15.8	64.90
Alfalfa ...	100	out-of-doors	check	C.F.	21.6	84.4	60.87
Alfalfa ...	100	out-of-doors	heat	0	2.1	120.0	39.73
Alfalfa ...	100	out-of-doors	toluene	F.	49.0	28.3	52.02
Alfalfa ...	100	20	check	C.F.	8.0	16.4	65.53
Alfalfa ...	100	20	heat	0	1.1	19.1	44.10
Alfalfa ...	100	20	toluene	F.	22.7	16.8	69.93
Alfalfa ...	100	30	check	C.F.	4.5	26.6	63.33
Alfalfa ...	100	30	heat	0	4.0	300.0	45.36
Alfalfa ...	100	30	toluene	F.	3.5	8.6	61.74
None	70	out-of-doors	check	C.F.	4.9	10.1	72.45
None	70	out-of-doors	heat	0	1.4	8.0	51.03
None	70	out-of-doors	toluene	0	8.0	4.9	73.71
None	70	20	check	C.F.	3.0	10.3	68.04
None	70	20	heat	0	0.3	12.1	40.95
None	70	20	toluene	0	4.4	4.1	77.03
None	70	30	check	C.F.	1.1	7.0	77.83
None	70	30	heat	0	0.15	8.0	83.38
None	70	30	toluene	F.	0.9	9.1	74.34
None	100	out-of-doors	check	C.F.	4.2	11.0	74.97
None	100	out-of-doors	heat	F.	1.0	11.5	78.12
None	100	out-of-doors	toluene	C.F.	5.0	10.5	74.86
None	100	20	check	C.F.	5.0	18.6	69.30
None	100	20	heat	0	1.5	7.4	54.55
None	100	20	toluene	F.	4.7	7.1	67.88
None	100	30	check	C.F.	1.1	4.5	75.00
None	100	30	heat	0	0.3	7.2	67.41
None	100	30	toluene	F.	1.6	4.2	74.36
Average ..	.....	.....	check	.....	8.3	33.0	69.14
Average ..	.....	.....	heat	.....	1.7	45.0	56.84
Average ..	.....	.....	toluene	.....	22.3	25.8	66.27

[1] A 28-day incubation period was used.

[2] Presence of protozoa, not their activity, was determined. C.—Ciliates; F.—Flagellates.

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tion are entirely different: the treated soils which at times gave the largest numbers of bacteria, frequently gave the smallest ammonia accumulation; the untreated and toluene-treated soils containing protozoa and fewer bacteria, gave higher ammonia accumulation.

These results are not in accord with Russell and Hutchinson's (8) conclusions. The presence of protozoa seems to check the bacterial numbers, but has no influence upon their ammonifying efficiency; as a matter of fact, the untreated soils containing ciliates and flagellates gave on the average slightly higher ammonia accumulation than the toluene-treated soils, which contained only flagellates. The ammonifying efficiency of a soil and its bacterial content do not seem to go hand in hand, but there appears to be a fair consistency. These results bear out the conclusions of the previous paper (12), that the protozoa seem to have a detrimental effect upon the numbers of bacteria, but not upon their ammonifying efficiency.

Of course, this method of attacking the problem of interrelationship between the protozoa and bacteria in the soil is open to severe criticism, since this study has been made on sterilized soil, where the conditions are absolutely non-comparable with ordinary soils. Not only the presence of protozoa, but also their active condition was demonstrated in all instances when inoculated into sterilized soil, if the organic matter and moisture content were high enough for them to be active. If a study is made merely of the two groups of organisms, not of the natural soil conditions, the sterilized soil affords a good means of doing it, unless there may be groups of protozoa (perhaps the harmful ones), which would not be active under these conditions. The whole problem in then changed, and we do not have to speak of the influence of protozoa, as a group, upon bacteria, but of certain organisms, which may not be protozoa at all, or which develop only when soil is kept under abnormal conditions.

The fact that the soils containing the full moisture-holding capacity, in which active protozoa were found in most examinations, gave lower bacterial counts and lower ammonifying efficiency than the soils with the optimum moisture, in which protozoa were not found to be active, might be used for the assertion that the protozoa act injuriously upon the bacteria and their activities in the soil. The same thing would hold true with the different temperatures of incubation. The higher the moisture content and the higher the temperature of incubation (up to a certain limit), the more active the protozoa are found to be. But these conditions give also lower bacterial numbers and lower ammonifying efficiency. Hence, if one would correlate the two it would look as if the protozoa produce that effect. But anyone who studies environmental conditions of organisms would say that increasing the moisture content above the optimum and the temperature above normal will lessen the vitality of the organisms, hence the decrease in bacterial numbers and their ammonifying effi-

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ciency. By making the conditions abnormal the vitality of the bacteria is lessened, but these conditions seem to favor protozoan activity, and it was only natural for Russell and Hutchinson (7, 8) to correlate the two. Antiseptics seem to improve the conditions for bacterial activities; whether it is due to the killing of the less vital bacteria and large types of protozoa, thus producing a large amount of easily available organic matter; or to the destruction of soil toxins that have been formed under the abnormal conditions; or to the destruction of harmful organisms which might be protozoa, but not all the protozoa, since not all of them are destroyed; or to the improvement of conditions for other organisms, such as fungi, which would otherwise not be very active; or, finally, to all of those causes together and perhaps an entirely different one,—nothing definite can be said as yet.

To be able to get an idea, how partial sterilization of the soil influences not only the microörganisms in the soil, but the soil itself with the microorganisms in it, the Penn loam soils were treated differently, as has been outlined in the methods above.

Comparing the results obtained in Table III, it is seen that the action of heat and toluene have a beneficial effect upon the ammonia accumulated in the soils. While the average of the ammonia accumulated in 30-gm. portions of 16 untreated soils was 3.64 mg. of nitrogen, that accumulated in the corresponding portions treated by heat was 6.41 mg. N., and in those treated by toluene 4.43 mg. N. The same thing holds true with the bacterial numbers: the untreated soils contained on the average 8,600,000 of bacteria per gram, this number dropping to 4,100,000 in 30 days. The heat treatment reduced the bacterial numbers from 8,600,000 to 1,760,000

TABLE III

EFFECT OF HEAT AND TOLUENE ON BACTERIAL NUMBERS AND AMMONIA PRODUCTION IN PENN LOAM SOIL

Soil Conditions				Millions of Bacteria per gram of Soil		Ammonia Produced in the Soil, in mg. N.
Manure	Moisture %	Temperature ° C	Treatment	At the Beginning	After 30 Days	
Stable	70	out-of-doors	check	1.6	1.7	1.28
Stable	70	out-of-doors	heat	0.2	2.6	0.64
Stable	70	out-of-doors	toluene	0.9	5.0	2.30
Stable	70	20	check	6.2	3.5	0.77
Stable	70	20	heat	0.2	45.6	4.35
Stable	70	20	toluene	1.9	33.7	2.82
Stable	70	30	check	3.7	4.2	0.13
Stable	70	30	heat	0.6	6.6	0.90
Stable	70	30	toluene	3.9	17.8	1.66
Stable	100	out-of-doors	check	9.0	3.2	2.56
Stable	100	out-of-doors	heat	0.2	14.9	1.92
Stable	100	out-of-doors	toluene	3.0	14.2	1.79

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TABLE III—Continued

EFFECT OF HEAT AND TOLUENE ON BACTERIAL NUMBERS AND AMMONIA PRODUCTION IN PENN LOAM SOIL

Soil Conditions				Millions of Bacteria per gram of Soil		Ammonia Produced in the Soil, in mg. N.
Manure	Moisture %	Temperature ° C.	Treatment	At the Beginning	After 30 Days	
Stable	100	20	check	7.7	2.2	0.38
Stable	100	20	heat	0.7	17.7	5.23
Stable	100	20	toluene	2.4	9.0	4.10
Stable	100	30	check	20.6	1.7	1.15
Stable	100	30	heat	15.8	10.4	4.22
Stable	100	30	toluene	15.9	26.7	4.61
Alfalfa	70	out-of-doors	check	19.9	5.6	18.18
Alfalfa	70	out-of-doors	heat	1.9	18.9	24.58
Alfalfa	70	out-of-doors	toluene	1.2	14.5	19.20
Alfalfa	70	20	check	7.7	8.4	11.52
Alfalfa	70	20	heat	0.3	8.9	29.18
Alfalfa	70	20	toluene	9.9	10.2	16.13
Alfalfa	70	30	check	41.3	16.0	12.42
Alfalfa	70	30	heat	4.7	7.3	16.13
Alfalfa	70	30	toluene	35.5	39.5	3.84
Alfalfa	100	20	check	11.5	8.0	6.78
Alfalfa	100	20	heat	0.6	14.0	8.06
Alfalfa	100	20	toluene	8.4	20.2	8.06
None	70	out-of-doors	check	1.5	1.0	0.00
None	70	out-of-doors	heat	0.7	2.9	1.15
None	70	out-of-doors	toluene	0.6	10.6	1.28
None	70	20	check	0.9	1.6	1.02
None	70	20	heat	0.2	2.2	0.90
None	70	20	toluene	0.6	12.4	0.26
None	70	30	check	1.2	2.4	0.13
None	70	30	heat	0.6	3.8	1.02
None	70	30	toluene	1.0	12.2	1.40
None	100	out-of-doors	check	1.9	2.5	0.38
None	100	out-of-doors	heat	0.8	4.1	1.28
None	100	out-of-doors	toluene	2.0	25.6	0.90
None	100	20	check	0.3	1.1	0.64
None	100	20	heat	0.2	2.8	1.02
None	100	20	toluene	0.8	3.2	0.68
None	100	30	check	3.1	3.1	0.90
None	100	30	heat	0.6	18.4	2.04
None	100	30	toluene	1.1	12.5	1.92
Average	.....	.....	check	8.6	4.1	3.64
Average	.....	.....	heat	1.8	11.3	6.41
Average	.....	.....	toluene	5.6	16.7	4.43
Average	.....	out-of-doors	.....	[1]6.8	8.5	5.16
Average	.....	20	.....	[1]5.4	11.4	5.27
Average	.....	30	.....	[1]9.2	12.2	3.50
Average	70	*	.....	[1]9.3	11.1	....
Average	100	.....	.....	[1]7.7	10.3	....

[1] Only checks were used for these averages; in all other cases all soils, treated and untreated, were used for the average.

* Average for ammonia is not given, since the alfalfa soils which gave the largest amounts of ammonia were present in 3 cases for the 70 per cent moisture and only in one case for the 100 per cent. Hence a true average would not be obtained.

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per gram; but after proper moisture had been added and the soils allowed to incubate for 30 days, the numbers increased to 11,300,000, almost three times that of the untreated soils. The treatment with toluene also lowered the bacterial numbers; after 30 days' incubation the numbers increased from 5,600,000 to 16,700,000 per gram of soil, more than four times the numbers found in the untreated soils.

This experiment confirms fully the results of Russell and Hutchinson (7, 8) that partial sterilization of soil by heat and antiseptics increases the amount of ammonia produced in the soil and also the bacterial numbers; but this is not due to the destruction of protozoa, since in the case of the toluene treated soils some of the protozoa, namely the flagellates and in some cases small ciliates, were left, while there was an increase in ammonia production and in bacterial numbers. However, the idea expressed by Dr. Löhnis that there might be two kinds of protozoa in the soil, one injurious to bacteria and thus to soil fertility and another noninjurious and perhaps even beneficial, may hold true.

The action of toluene and heat is greatest upon the soils having a high content of undecomposed organic matter; the presence of protozoa does not seem to have any important part to perform here, because the action of antiseptics and heat will be much less in soils having a low content of organic matter, though it may contain protozoa. The reason why most investigators have failed to get results similar to those obtained by Russell and his associates may be due to the fact that they did not work with soils of such a high content of organic matter as Russell and his associates did.

Attention should be called to the fact that in many instances where the soil has been treated with heat and toluene, the fungi were found at the end of 30 days' incubation in much larger numbers than in the checks; since these organisms, as was shown in another place (11), are very strong ammonifyers, the increase in ammonia production may be due not at all to the increase in bacterial numbers, but to the improved conditions for fungus activities. The rapid decomposition of the organic matter by these organisms may lead to increased bacterial activities, so that the beneficial results of the partial sterilization may not be due at all to the destruction of some protozoa, but to the improvement of the soil conditions for the action of microörganisms, such as fungi, which otherwise remain in a not very active stage.

Summary

1. The presence of protozoa seems to check the bacterial numbers, which are found to be smaller in the soils where the protozoa are present than in the corresponding soils where they are absent.

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2. The ammonifying efficiency of the soil does not go hand in hand with the changes in bacterial numbers.

3. Heating the soil at 65° C. for 5 hours destroyed the protozoa in all instances but one, and greatly reduced the bacterial numbers; but when proper moisture was added and the soils were allowed to incubate for 30 days, the bacterial numbers increased to almost three times those of the check. There was a corresponding increase in ammonia production in the soil.

4. The action for 48 hours of 4 per cent toluene, which was then allowed to evaporate for 48 hours, killed the ciliates, but not the flagellates; this treatment also reduced the bacterial numbers, but they at once increased after the toluene was allowed to evaporate.

5. The action of toluene and heat is greatest upon soils having a high content of organic matter, whether the protozoa are active or not.

6. The Sassafras soils kept out-of-doors gave higher bacterial numbers and higher ammonifying efficiency than those kept under laboratory temperatures and the latter gave higher numbers and ammonifying efficiency than those kept at 30° C.

7. The soils with the optimum moisture, gave in the main, higher bacterial numbers and ammonifying efficiency than those containing full moisture-holding capacity.

8. There may be two types of protozoa in the soil, one injurious to bacteria and to soil fertility, and the other uninjurious and perhaps even beneficial; the beneficial influence of antiseptics upon soil may be due to the killing of the first type of protozoa.

9. There are, however, other factors, which point out that the improved soil conditions brought about by the action of heat and antiseptics may be due to the improvement of conditions for other organisms, such as fungi, to work in the soil; or the soil itself may be changed in such a manner as to offer new conditions to its microörganic population.

Literature Cited

(1) Cunningham, Andrew.
1915. Studies on soil protozoa. *In* Jour. Agr. Sci., v. 7, p. 49-74.

(2) Gainey, P. L.
1912. The effect of toluol and CS_2 upon the microflora and fauna of the soil. *In* Mo. Bot. Gard. Ann. Rpt., v. 23, p. 147-169.

(3) Greig-Smith, R.
1912. The inactivity of the soil protozoa. *In* Proc. Linn. Soc. N. S. Wales, v. 37, pt. 4, p. 655-672.

(4) Hiltner, L., and Sörmer, K.
1903. Studien über die Bakterienflora des Ackerbodens, mit besonderer Berucksichtigung ihres Verhaltens nach einer Behandlung mit Schwefelkohlenstoff und nach Brache. *In* Arb. K. Gsndhtsamt., Biol. Abt., Bd. 3, p. 445-528.

Digitized by Google

(5) RUSSELL, E. J., and BUDDIN, W.
1913. The action of antiseptics in increasing the growth of crops in soil. *In* Jour. Soc. Chem. Indus., v. 32, p. 1136-1142.

(6) RUSSELL, E. J., and GOLDING, J.
1912. Investigations on "sickness" in soil. I. Sewage sickness. *In* Jour. Agr. Sci., v. 5, p. 27-47.

(7) RUSSELL, E. J., and HUTCHINSON, H. B.
1909. The effect of partial sterilization of soil on the production of plant food. *In* Jour. Agr. Sci., v. 3, p. 111-144.

(8) RUSSELL, E. J., and HUTCHINSON, H. B.
1913. The effect of partial sterilization of soil on the production of plant food. II. The limitation of bacterial numbers in soils and its consequences. *In* Jour. Agr. Sci., v. 5, pt. 2, p. 152-221.

(9) RUSSELL, E. J., and PETHERBRIDGE, F. R.
1912. Investigations on "sickness" in soil. II. Sickness in glasshouse soils. *In* Jour. Agr. Sci., v. 5, p. 86-111.

(10) SHERMAN, J. M.
1916. Studies on soil protozoa and their relation to the bacterial flora. *In* Jour. Bact., v. 1, p. 35-66, 165-185.

'11) WAKSMAN, S. A.
1916. Soil fungi and their activities. *In* Soil Sci., v. 2, p. 103-155.

(12) WAKSMAN, S. A.
1916. Studies on soil protozoa. *In* Soil Sci., v. 1, p. 135-152.

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THE LOESS SOILS OF THE NEBRASKA PORTION OF THE TRANSITION REGION:

V. THE WATER-SOLUBLE CONSTITUENTS[1]

By

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The following paper presents the results of an investigation of the water-soluble material of the loess soils of the Nebraska portion of the transition region. The general chemical and physical properties of these soils have been dealt with in detail, in papers by Alway, et al (1, 2, 3, 4).

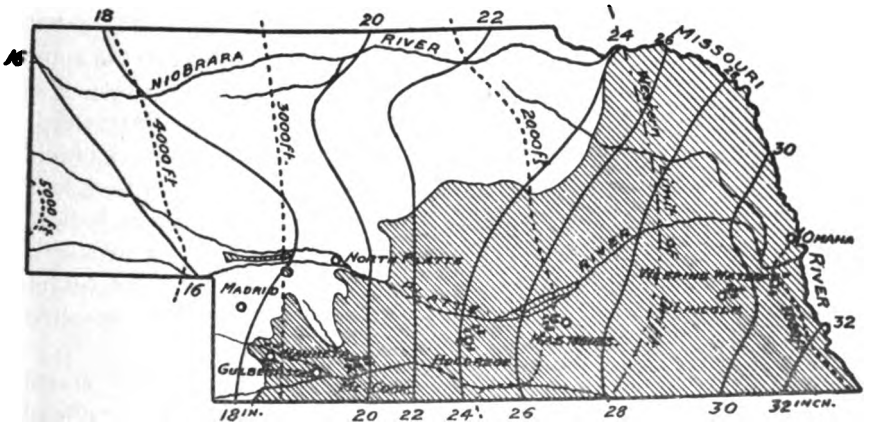

Fig. 1.—Map of Nebraska showing distribution of the loess, precipitation belts, the altitude and the locations of the fields sampled.

In Nebraska the loess soils extend westward from the Missouri river a distance of about 300 miles. Throughout this region the temperature conditions are quite uniform but there is a gradual decrease in the humidity of the climate. The normal annual precipitation which exceeds 30 inches at the eastern boundry, falls steadily until it is less than 20 inches in the extreme western portion, while the rate of evaporation increases considerably. The climate of this region is described in detail by Alway and McDole (3, p. 239).

[1] Received for publication July 22, 1916.

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The soil samples which were used in carrying out the work here reported were collected by Alway and McDole and are the same samples on which the article, above referred to, is based. They were collected from 30 virgin prairie fields, five near each of six stations of the Weather Bureau of the U. S. Department of Agriculture, as shown in the map (fig. 1)—Wauneta, McCook, Holdrege, Hastings, Lincoln and Weeping Water. In each field at intervals of 30 feet, ten borings were made to a depth of 6 feet and composite samples prepared of each foot section, thus giving six samples from each field, the so-called "field samples." From these the "area samples" were prepared by mixing equal weights of the corresponding five "field foot-samples." Thus each of the "area foot-samples" is a composite from 50 individual borings. The method of sampling is described in detail in the article above referred to.

Determinations of total solids, non-volatile inorganic material, carbonic acid, bicarbonic acid, chloride ion, phosphate ion, sulphate ion, potassium, calcium and magnesium ions were made on each of the "area foot-samples." The methods employed were those described by Schreiner and Failyer (6). The solution was obtained by shaking 200 gm. of soil with 500 c.c. of carbon dioxide-free distilled water at 5-minute intervals for 2 hours and allowing to stand over night. The solution was filtered through a Chamberland-Pasteur porcelain tube in the apparatus devised by Briggs (5, p. 31). Total solids were obtained by evaporation of 100 c.c. of this solution to dryness and heating to constant weight at 110° C. Non-volatile inorganic material was determined by ignition of the total solids. Bicarbonate was determined by titration with standard potassium hydrogen sulphate solution, using methyl orange as indicator. Chlorides were determined in the usual way by titration with standard silver nitrate. Sulphates and calcium were determined by the turbidity method (6, p. 19) and phosphates, potash and magnesium by the colorimetric methods as described by Schreiner and Failyer (6, p. 53).

It should be emphasized that the determination of such small amounts of material as are present in water extracts of ordinary soils is attended with unusual experimental difficulties. In the work here reported every precaution was taken to insure the highest attainable degree of accuracy. Those reagents which were liable to become contaminated through contact with glass were stored in paraffin containers. In the determination of any one constituent the same procedure was followed throughout. Colorimetric and turbidity methods are open to criticism because the personal equation of the operator is an important factor. Furthermore some of the estimations by these methods are in themselves only approximations, whereas some have been so highly perfected that they surpass gravimetric estimations in accuracy. In the estimation of small amounts of material such as are found in soil extracts, one is left no choice as to methods. The colorimetric and turbidity methods used in this work ad-

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mit of the determination of small amounts of material such as could not possibly be detected by ordinary gravimetric methods. Thus while the absolute accuracy of some of the results here reported may be questioned, nevertheless we feel that the results are of comparative value and in this respect have a real meaning.

In Table I are given the results of the analyses of the extracts of the "foot samples" from the six different areas. Results are expressed as parts per million of dry soil.

An inspection of the tables brings out first of all the fact that the soils of the Nebraska loess are remarkably uniform as regards their content of water-soluble material. On the whole the study indicates that soils with enough water-soluble material to be regarded as "alkaline" are nowhere to be expected on the prairies of the transition region, except possibly in depressions. The highest amount of total water-soluble material occurs in the first foot of the Lincoln area and the smallest amount in the sixth foot of the Weeping Water area. The bicarbonates seem responsible for the larger part of the inorganic material.

In Table II are given the figures of Table I rearranged for better comparison.

Inorganic Material

As regards total inorganic material the largest amount is found in the sixth foot of the Wauneta area and the smallest amount in the fifth foot of the Weeping Water area. In all the areas excepting the Weeping Water the total inorganic material increases somewhat from the first foot downward, though not in a regular way. The average amount of inorganic material in the six foot-sections decreases quite regularly as we pass from west to east. This is accounted for by the greater rainfall in the eastern areas and the consequent greater amount of leaching.

Bicarbonates

The water-soluble carbon dioxide appeared in the form of bicarbonates entirely, no carbonates being found in the extracts of any of the soils. The water-soluble bicarbonates increase quite regularly from the first foot downward in all of the areas, excepting the Weeping Water. In each of the foot sections they decrease quite regularly as we pass from west to east. They are highest in the third foot of the Hastings area and lowest in the lower sections of the Weeping Water area, being practically zero in the sixth foot. The average amount of bicarbonate decreases quite regularly from west to east, except that in the Hastings area it is slightly higher than in the Holdrege area. Bicarbonates follow the total inorganic material in this respect. The variation in the bicarbonate agrees in general with that of the total carbon dioxide as found by Alway and Rost, (4, p. 114) which averaged 1.49 per cent in the McCook area

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TABLE I

WATER-SOLUBLE CONSTITUENTS FROM FOOT SAMPLES OF LOESS SOILS EXPRESSED IN PARTS PER MILLION

Depth Foot	Total solids	Inorganic	HCO_3	Cl	K	PO_4	SO_4	Ca	Mg
WAUNETA AREA									
1	598	213	173	17	38	20	9	30	8
2	415	203	182	16	36	14	41	24	8
3	310	138	186	6	60	12	2	20	6
4	358	233	195	14	87	5	34	14	3
5	365	253	225	15	71	6	45	17	4
6	398	298	247	21	71	5	64	17	4
McCOOK AREA									
1	533	150	134	Trace	49	14	Trace	24	7
2	463	195	270	4	60	8	Trace	31	13
3	370	218	225	8	73	9	8	21	6
4	308	183	222	13	60	8	33	11	3
5	378	255	213	21	61	14	71	9	2
6	470	243	206	34	58	10	98	12	2
HOLDREGE AREA									
1	613	202	107	52	51	17	19	22	10
2	390	145	62	18	36	16	38	14	5
3	370	238	244	9	49	21	27	22	13
4	308	175	188	15	53	26	6	29	6
5	313	168	178	12	41	22	29	22	5
6	313	213	177	16	56	23	36	18	4
HASTINGS AREA									
1	730	140	95	54	60	10	9	29	12
2	365	83	47	20	31	8	39	15	6
3	440	210	328	13	52	7	29	31	17
4	370	235	261	18	53	8	30	26	11
5	353	185	210	17	49	6	29	21	8
6	293	170	202	21	44	6	28	16	6
LINCOLN AREA									
1	1188	265	113	36	38	22	41	24	23
2	565	140	54	18	20	13	45	14	11
3	360	95	57	19	18	11	51	10	6
4	425	250	165	28	18	6	83	20	16
5	343	208	194	23	18	6	42	15	12
6	320	205	170	21	20	7	43	12	9
WEEPING WATER AREA									
1	885	250	88	16	23	12	38	16	10
2	665	155	47	8	17	10	9	10	5
3	328	107	26	10	11	10	13	3	3
4	140	45	9	7	7	12	20	Trace	1
5	110	40	11	8	6	12	18	Trace	1
6	95	85	..	15	11	..	23	..	1

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and only 0.02 per cent in the Weeping Water area. Again, the lesser amount of leaching accompanying the lower rainfall would account for the much higher bicarbonate content in the soils of the western areas as compared with those of the eastern.

Chlorides

The water-soluble chlorides are low in all the soils of all the areas and account for but a small portion of the total soluble inorganic material. There is nothing noteworthy about their distribution. Excepting the first foot of the Hastings and Holdrege areas, the distribution of chlorides is quite uniform both as to depth and area.

Potassium

The distribution of the water-soluble potassium is noteworthy. It is highest in the Wauneta and McCook areas and lowest in the Weeping Water area, the average for the six foot-sections being nearly five times as great in the former as in the latter. In the extreme eastern area, the potassium *decreases* in passing from the first to the sixth foot, whereas in the Wauneta area it *increases* from the first foot downward. The intervening areas show an intermediate behavior. The potash in these same soils, soluble in 1 per cent citric acid, was found by Alway and Isham (2, p. 307) to exhibit a similar behavior. On the average it was practically three times as high in the western as in the eastern area. Furthermore it was found to *decrease* from the surface foot downward in the Weeping Water area, the figures being 0.036 per cent and 0.016 per cent for the first and sixth feet respectively with an average of 0.025 per cent for the six feet; whereas in the Wauneta area the citric-acid-soluble potash *increased* from 0.047 per cent in the surface foot to 0.083 per cent in the sixth foot, with an average of 0.071 per cent for the six feet. The intervening areas showed an intermediate behavior as regards their content of potash, soluble in 1 per cent citric acid. This peculiarity in the distribution of the water-soluble and citric-acid-soluble potassium is in striking contrast to the distribution of the total potassium and the potassium soluble in strong acids. Alway and Isham (2, p. 301) found the total, as well as the strong acid-soluble potash, to be quite uniformly distributed in these soils. The average total potash for all the sections of all the areas was 2.54 per cent and in none of the sections of any of the areas does the total potash vary more than 0.21 per cent from this figure. Similar results were obtained for the potash soluble in strong hydrochloric acid. It would seem that the same compounds which are responsible for the citric-acid-soluble potash, are responsible also for the water-soluble potash in these soils.

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TABLE II

WATER-SOLUBLE CONSTITUENTS FROM FOOT SAMPLES OF LOESS SOILS EXPRESSED IN PARTS PER MILLION

Constituents	Depth	Area					
		Wauneta	McCook	Holdrege	Hastings	Lincoln	W. Water
Inorganic .	1	213	150	202	140	265	250
	2	203	195	145	83	140	155
	3	138	218	238	210	95	108
	4	233	183	175	235	250	45
	5	253	255	168	185	208	40
	6	298	243	213	170	205	85
Average ..		223	207	190	170	193	113
HCO_3	1	173	134	107	95	113	88
	2	182	270	62	47	54	47
	3	185	225	244	328	57	26
	4	195	222	188	261	165	9
	5	225	212	178	210	194	11
	6	247	205	177	202	170	..
Average ..		201	211	159	190	125	30
Cl	1	17	..	52	54	36	16
	2	16	4	18	20	18	8
	3	6	8	9	13	19	10
	4	14	13	15	18	28	7
	5	15	21	12	17	23	8
	6	21	34	16	21	21	15
Average ..		15	13	20	24	24	11
K	1	38	49	51	60	38	23
	2	36	60	36	31	20	17
	3	60	73	49	52	18	11
	4	87	60	53	53	18	7
	5	71	61	41	49	18	6
	6	71	58	56	44	20	11
Average ..		60	60	48	48	22	12
PO_4	1	20	14	17	10	22	12
	2	14	8	16	8	13	10
	3	12	9	21	7	11	9
	4	5	8	26	8	6	12
	5	6	14	22	6	6	12
	6	5	10	23	6	7	..
Average ..		10	11	21	7	11	11

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TABLE II—(Continued)

WATER-SOLUBLE CONSTITUENTS FROM FOOT SAMPLES OF LOESS SOILS EXPRESSED IN PARTS PER MILLION

Constituents	Depth	Area					
		Wauneta	McCook	Holdrege	Hastings	Lincoln	W. Water
SO_4	1	9	Trace	19	9	41	38
	2	41	Trace	38	39	45	9
	3	2	8	27	29	51	13
	4	34	33	6	30	83	20
	5	45	71	29	29	42	18
	6	64	98	36	28	43	23
Average ..		32	35	26	27	51	20
Ca	1	30	24	22	29	24	16
	2	24	31	14	15	14	10
	3	20	21	22	31	10	3
	4	14	11	29	26	20	Trace
	5	17	9	22	21	15	Trace
	6	17	12	18	16	12	..
Average ..		20	18	21	23	16	..
Mg	1	8	7	10	12	23	10
	2	8	13	5	6	11	5
	3	6	6	13	17	6	3
	4	3	3	6	11	16	1
	5	4	2	5	8	12	1
	6	4	2	4	6	9	1
Average ..		6	6	7	10	13	3
Volatile Matter (Organic)	1	385	383	411	590	923	635
	2	202	268	245	282	425	510
	3	172	152	132	230	265	220
	4	125	125	133	135	175	95
	5	112	123	145	168	135	70
	6	100	227	100	123	115	10
Average ..		182	213	194	255	339	257
Total solids	1	598	533	613	730	1188	885
	2	415	463	390	365	565	665
	3	310	370	370	440	360	328
	4	358	308	308	370	425	140
	5	365	378	313	353	343	110
	6	398	470	313	293	320	95
Average ..		407	420	384	426	533	370

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Phosphoric Acid

The water-soluble phosphates on the whole show no regularity in distribution. Considering the amount in the whole six-foot section, they do not increase from west to east as does the water-soluble potash but are similar in amount in the extreme areas, with a somewhat higher content in the central areas, notably in the Holdrege. In the most western area it shows a decrease from the surface downward, whereas in the other areas it shows no very regular distribution. The distribution of the water-soluble phosphoric acid as compared with the phosphoric acid, soluble in 1 per cent citric acid, does not show the striking similarity which is brought out by a comparison of the water-soluble and citric-acid-soluble potash. Alway and Isham (2, p. 307) have shown that the phosphoric acid, soluble in 1 per cent citric acid, is highest in the two central areas and lowest in the two extreme areas. Furthermore it decreased from the surface foot downward in the two most westerly areas, whereas in the extreme eastern area it very markedly increased from the surface downward. The water-soluble phosphoric acid agrees with this only in being somewhat higher in the Holdrege area and in showing some decrease from the surface downward in the Wauneta area. It would seem that the comparative uniformity in the distribution of the water-soluble phosphates is due to the fact that the phosphates of the soil are only slightly water-soluble and that there is present in all these soils more than enough to saturate the water solution.

Sulphates

The distribution of the water-soluble sulphates shows no significant variations. It is on the whole quite uniform, except that sulphates are practically absent in the upper sections of the McCook area and are somewhat higher in the Lincoln area than in any of the others.

Calcium and Magnesium

The water-soluble calcium is distributed very uniformly throughout the different areas except that it is practically absent in the lower levels of the Weeping Water area. Both the total and the acid-soluble calcium were found by Alway and Rost (4, p. 413) to vary with the area, being more than three times as high in the lower levels of the western areas, as in those of the eastern areas. They also increased from the surface foot downward in all the sections. The water-soluble magnesium is even more uniformly distributed than is the calcium and follows the total and acid-soluble magnesium, as determined by Alway and Rost, in this respect.

Volatile Matter

The water-soluble material which is volatile on ignition is chiefly organic matter, with some carbon dioxide from alkaline earth carbonates, and possibly some hydration water and water of constitution.

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In general, the distribution of the water-soluble volatile matter agrees with that of the total volatile matter and the organic carbon as determined by Alway and McDole (3, p. 228, 232). It increases quite regularly from west to east, especially in the upper three feet. It furthermore decreases regularly from the surface foot downward in all of the areas, and obviously, is higher in the eastern areas because of the greater amount of vegetative growth.

Total Soluble Material

The distribution of the total water-soluble material is quite uniform when the average of the six sections of the different areas is considered. In the upper sections it increases from west to east, whereas in the lower sections it decreases more or less uniformly. The greater amount of volatile organic matter in the upper sections of the eastern areas together with a greater amount of inorganic material in the lower sections of the western areas accounts for this variation.

Summary

In general the water-soluble material in the loess soils of the Nebraska portion of the transition region is low. The average total water-soluble material is quite uniformly distributed as to area, the inorganic material increases with the aridity, whereas the volatile material decreases with increasing aridity.

The water-soluble bicarbonates decrease from west to east and follow the total carbonates in this respect. The water-soluble chlorides and sulphates as well as calcium and magnesium are quite uniformly distributed and follow the distribution of the total amounts of these constituents, in these same soils.

The water-soluble potassium is highest in the western areas and lowest in the eastern areas. In the extreme eastern area it decreases from the surface downward, whereas in the western area the reverse is true. In this respect it follows the citric-acid-soluble potash and not the total potash, which is quite uniformly distributed.

The water-soluble phosphates show no very regular distribution.

Literature Cited

(1) Alway, F. J., and Blish, M. J.
1916. The loess soils of the Nebraska portion of the transition region: II Humus, humus-nitrogen and color. *In* Soil Sci., v. 1, no. 3, p. 239-258.

(2) Alway, F. J., and Isham, R. M.
1916. The loess soils of the Nebraska portion of the transition region: III Potash, soda and phosphoric acid. *In* Soil Sci., v. 1, no. 4, p. 299-316.

Digitized by Google

(3) ALWAY, F. J., and MCDOLE, G. R.
1916. The loess soils of the Nebraska portion of the transition region: I Hygroscopicity, nitrogen and organic carbon. *In* Soil Sci., v. 1, no. 3, p. 197-238.

(4) ALWAY, F. J., and ROST, C. O.
1916. The loess soils of the Nebraska portion of the transition region: IV Mechanical composition and inorganic constituents. *In* Soil Sci., v. 1, no. 5, p. 405-436.

(5) BRIGGS, L. J.
1902. Filtration of suspended clay from soil solutions. *In* U. S. Dept. Agr. Bur. Soils Bul. 19, p. 31-40.

(6) SCHREINER, O., and FAILYER, G. H.
1906. Colorimetric, turbidity and titration methods used in soil investigations. U. S. Dept. Agr. Bur. Soils Bul. 31, 60 p.

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ON THE DISTRIBUTION OF PHOSPHORUS IN A VERTICAL SECTION OF BLUEGRASS SOIL[1]

By

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The most important chemical action concerned in the disintegration of limestone rock to form soil is the combination of the free carbonic acid contained in natural waters with the calcium carbonate and magnesium carbonate of the limestone to form calcium and magnesium bicarbonate, substances soluble in water [$CaCO_3 + H_2CO_3 = CaH_2(CO_3)_2$]. Thus the calcium and magnesium carbonates, which compose the greater part of most limestones, may be dissolved and carried away by percolating water, leaving behind the less easily soluble constituents of the limestone, such as sand, clay and other silicates, ferric oxid, etc. It is this residue of nearly insoluble material which finally becomes converted into soil. In regions of abundant rainfall, such as our Bluegrass country, this disintegration of the limestone and addition of the residuum to the soil is going on almost continuously at the surface of the rock beneath the soil. The rain water, as it falls upon the ground, already contains a little dissolved carbon dioxide derived from the air. In sinking through the soil it acquires more from the oxidation of organic matter in the soil. While passing through the soil, the water takes up more or less soluble material until its downward passage is arrested by the underlying limestone. Here the water may be supposed to collect for a time and be in contact with the limestone long enough to become fully saturated with calcium carbonate before it finds its way to the surface of the ground, at some lower point, in the form of a spring. Such waters carry in solution, besides calcium and magnesium bicarbonate, appreciable quantities of sodium, potassium, iron, silica, sulfates, chlorids, nitrates and phosphates, showing that even the less soluble or soil-making substances are attacked to some extent. The amount of phosphate carried away by the water of springs has recently been studied by Mr. J. S. McHargue, of the Kentucky Agricultural Experiment Station, and reported upon in a paper read at the 1915 meeting of this Academy. Mr. McHargue's analyses show that this amount is relatively small, as compared with the amount of calcium and magnesium

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carbonate present in these waters. The average from the analyses of the water of six different springs in Fayette and Woodford Counties shows one part of tricalcium phosphate to about 100 parts of calcium and magnesium carbonates, taken together, the extremes being 1:71, and 1:133. This phosphate may have been taken up either from the soil through which the water has percolated or from the limestone with which it has come in contact, or both. The relatively small solubility of tricalcium phosphate leads to the inference that, when a highly phosphatic limestone is acted upon by natural waters, the greater part of the phosphate contained in the limestone will be left in the resulting soil, after practically all the calcium carbonate has been dissolved away. Thus, if there is in a given limestone more than one part of tricalcium phosphate to 100 parts of calcium and magnesium carbonates, the excess of phosphate would remain undissolved and be added to the soil. The distribution of phosphorus in a column of soil, however, must be affected by the phosphate continuing to go into solution after all carbonate has been removed, thus reducing the amount in the upper part of the column. Again, in a very phosphatic soil, the fact that the percolating water has an opportunity to become saturated with phosphate before reaching the bed rock would greatly diminish or wholly nullify its solvent action upon the phosphate of the underlying limestone, thus tending all the more to preserve in the newly made soil at the bottom of the column the differences in phosphate content existing in the original limestone.

There are numerous analyses which show that the several layers of the limestone underlying the soil in the vicinity of Lexington differ greatly with regard to their phosphorus content. Published analyses give percentages of phosphorus ranging from 0.3 or less to about 14 per cent, the latter being in a very thin layer of limestone. The ratio of tricalcium phosphate to calcium and magnesium carbonate ranges from 1:60 to 1:1, or, from 1 2/3 parts to about 100 parts of phosphate to every 100 parts of carbonate. The natural inference from these facts and the considerations already stated is that when such limestones, lying in horizontal strata, are converted into soil by natural processes, there would result a soil having horizontal bands of very different phosphorus content, corresponding in a rough way to the several layers of the original limestone, and that these differences would be best preserved in the lower part of the column where the soil is comparatively new.

A series of samples, taken vertically through such a soil, should contain very different proportions of phosphorus. To test this hypothesis, several borings were made on the Experiment Station farm at Lexington, some years ago, by the writer, assisted by Mr. Robert Hart. The soil from each six inches was stored separately and analyzed for phosphorus by Mr. O. M. Shedd, of the Experiment Station. A graphical representation of results of two of these borings is presented herewith. The dif-

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ferences shown are just such as would be expected in a soil derived from the highly phosphatic layers of the Lexington limestone and they appear to even a more marked degree than was anticipated. It may be added that small pieces of nearly pure, soft phosphate could be distinguished in several of the samples.

It has been argued that some of the phosphate taken by the soil water from the upper part of the soil column may be deposited again when the lower part of the column is reached, where calcium carbonate is still present in effective quantity. The fact that tricalcium phosphate is less soluble in carbonated water in the presence of calcium carbonate than it is if calcium carbonate is absent, would favor this view. Such a process would tend to produce a maximum of phosphate at a short distance above the bed rock, as was found in the first boring; it would hardly account for the existence of two maxima, as found in the second boring. Besides, the large percentages and the magnitude of the differences found suggest strongly that the distribution of the phosphate in the limestone before it became soil, is the controlling factor, even if leaching and redeposition of phosphate has played some part. The fact that plates of phosphate similar to those occurring in the limestone may be found in the more phosphatic parts of the soil column supports this view.

It should be stated in this connection, also, that the amount of calcium carbonate in this soil is small. Analyses by Dr. Blumenthal of this Station on the soil from the first boring show the maximum of 0.135 per cent of calcium carbonate in the third six inches above the rock and the minimum 0.020 per cent in the fourth sixth inches from the top.

In comparing the two diagrams, it is important to bear in mind that the first boring was made near the top of a gentle slope, whereas, the second was near the bottom of such a slope, a slight rise intervening between the two. No doubt some of the older and well leached soil has been removed from the top of the first column, a condition which would account for the high percentage of phosphorus in the first foot, and that soil of a similar character has been added to the top of the second column, thus keeping down the phosphorus content in the upper four feet.

Both borings were started on nearly level ground at approximately the same elevation above sea level, namely, between 990 and 995 feet, as nearly as could be judged from the 5-foot contour lines on the map of the farm.

These considerations suggest that the maximum phosphate in the first diagram and the first maximum in the second should be referred to the same highly phosphatic beds in the original limestone.

It may not be without interest in this connection to speculate a little upon the character and thickness of a section of limestone capable of producing a soil section similar to that described, though it would be impossible to arrive at a definite or exact conclusion, from the data available. Of

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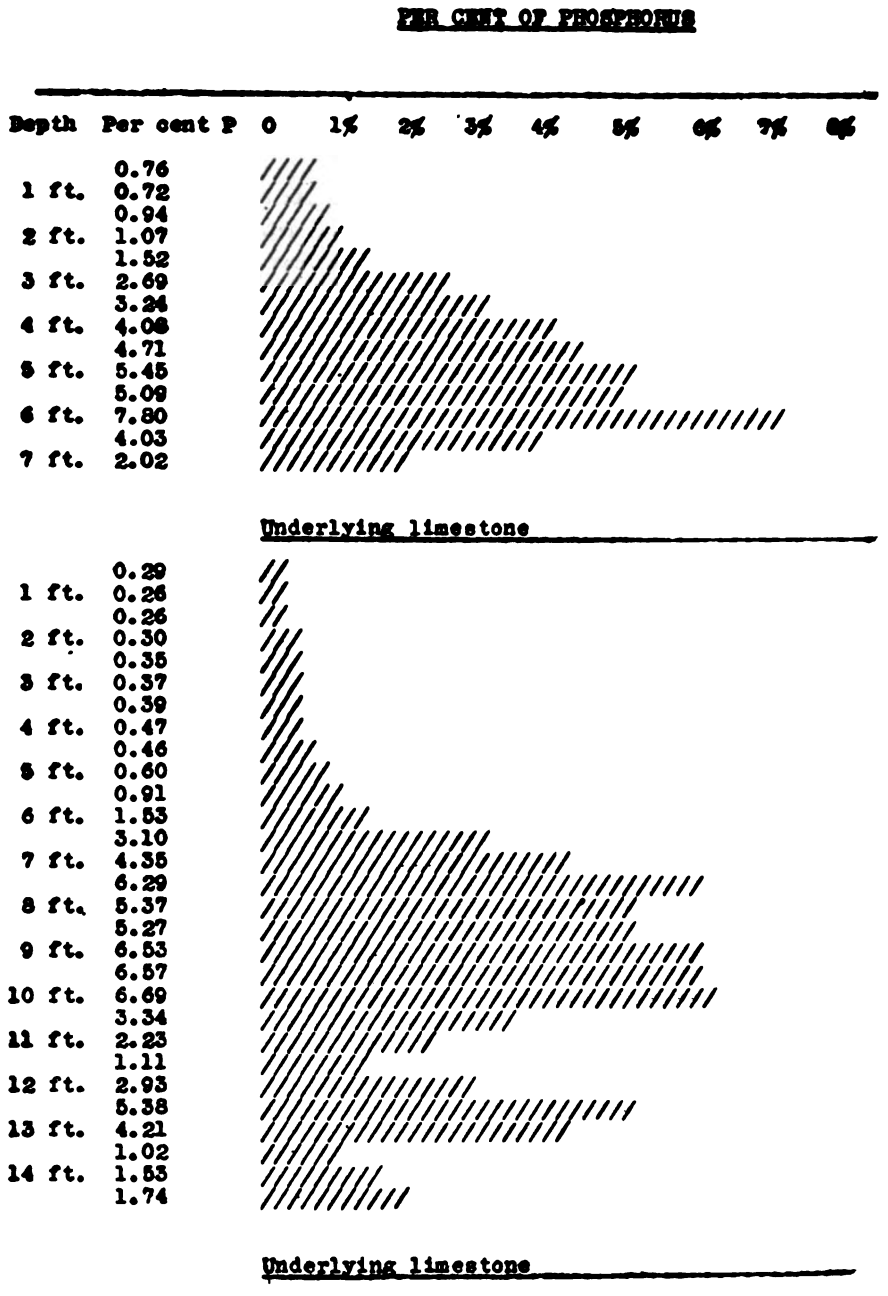

Fig. 1.—Phosphorus in soil sections, Experiment Station Farm, Lexington, Ky.
Top, Rose St. field, top of gentle slope.
Bottom, forage plots, bottom of gentle slope.
Elevation of the top of each, between 990 and 995 ft. above sea level. The two points are about 250 yards apart.

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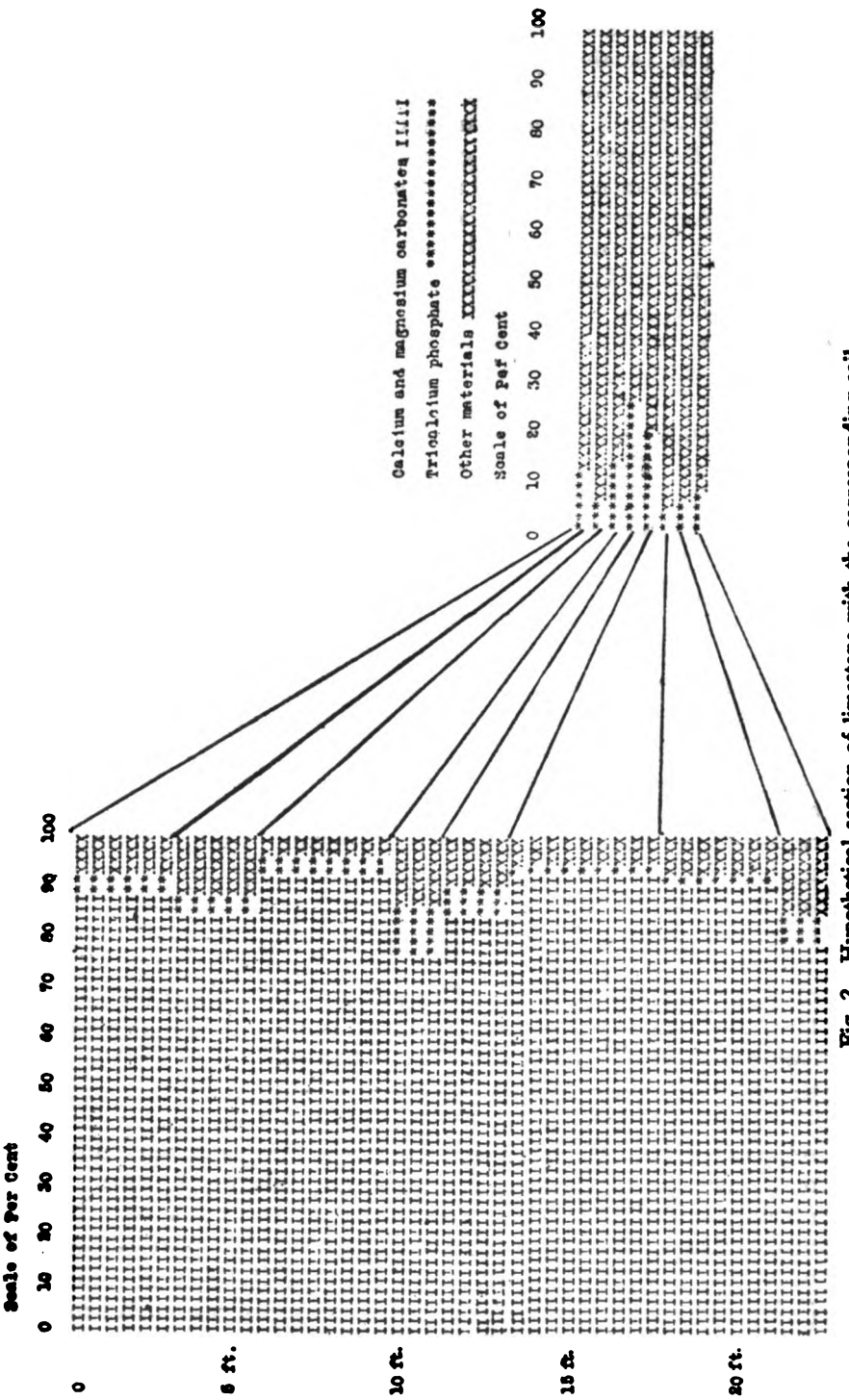
Scale of Per Cent
0 10 20 30 40 50 60 70 80 90 100
0
5 ft.
10 ft.
15 ft.
20 ft.
Calcium and magnesium carbonates IIIIII
Tricalcium phosphate **********
Other materials XXXXXXXXXXXXXXXX
Scale of Per Cent
0 10 20 30 40 50 60 70 80 90 100

Fig. 2.—Hypothetical section of limestone with the corresponding soil.

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great assistance in such a speculation would be a series of separate analyses of each consecutive layer in a vertical section covering a considerable thickness of the more phosphatic part of the Lexington limestone. Unfortunately, no such series of analyses has been made. In the chemical reports of the Kentucky Geological Survey, Dr. Robert Peter published phosphate determinations upon samples of limestone taken from several different layers exposed in the face of the McMeekin quarry on the Newtown road 4 miles north of Lexington, where he first discovered the phosphate, but the thickness of only one layer was reported. This was the principal phosphatic layer, about a foot thick, appearing at the bottom of the quarry face. Analyses of a sample from this layer gave 45.9 per cent of tricalcium phosphate, 49.2 per cent of calcium carbonate, including a little magnesium carbonate, and 4.9 per cent of other materials. The analyses of eleven other samples selected from different parts of this quarry face gave amounts of tricalcium phosphate ranging from 11.0 per cent to 47.9 per cent, the last being from a thin layer. It is evident that, in taking these samples, the more phosphatic layers were selected. Interspersed with these there must have been layers with a much smaller phosphate content. As the amount of carbonate in the other samples was not determined, we will try to estimate the result of the action of carbonated water upon the 12-inch layer of phosphatic limestone first mentioned. During the solution and removal of 49.2 parts of calcium carbonate by the process heretofore described, about 0.5 part of tricalcium phosphate will have been dissolved. Deducting this from the 45.9 parts present leaves 45.4 parts of tricalcium phosphate, with which will remain the 4.9 parts of other materials belonging to the limestone, making 50.3 parts of residuum, containing 90 per cent of tricalcium phosphate. This would be a high-grade phosphate, somewhat better than the richest plates of phosphate which have been picked out of the highly phosphatic soil corresponding to the geological horizon in which this quarry is situated.

We have here supposed a case where the solvent action on the phosphate was arrested as soon as all calcium carbonate had been removed. Under natural conditions, leaching would continue indefinitely, removing both phosphate and other materials and probably lowering the relative amount of phosphate in the residue considerably.

To obtain a residue more of the nature of soil, we may suppose a limestone containing less phosphate, more calcium carbonate and more of the other materials. For example, with 6.5 per cent of phosphate, 78.5 of calcium carbonate and 15.5 of other materials, the residue from 100 parts of limestone would consist of (6.5—.785) 5.715 parts of tricalcium phosphate and 15.5 parts of other materials, or 21.215 parts, containing $\left(\frac{5.715 \times 100}{21.215}\right)$ about 27 per cent of tricalcium phosphate, equivalent to 5.3

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per cent of phosphorus, or about the same as was found in the fifth six inches from the bottom of the second soil section.

To estimate how much of such a limestone would be required to produce a foot of soil, it is necessary to take into consideration the relative weights of limestone and soil. A cubic foot of limestone weighs about 163 pounds and a cubic foot of soil, about 92 pounds. The weight of a cubic foot of limestone, multiplied by the weight of undissolved phosphate and other materials from one pound of limestone ($163 \times .21215$) gives the weight of residuum from a cubic foot of the stone (34.58). Dividing the weight of a cubic foot of soil, by this figure $\left(\frac{92.}{32.58}\right)$ gives the number of cubic feet of limestone required to produce a cubic foot of soil (2.7 cu. ft.) The same figures will represent the thickness of limestone, in feet, necessary to produce one foot of soil on equal areas.

Making the calculation, the result is found to be 2.7 cubic feet; or, it would require approximately a foot and a half of this stone to produce the 6-inch layer of soil in the soil section referred to. The second diagram shows a hypothetical section of limestone which would produce layers of soil corresponding to those found in the lowest four feet of the second section. This part was selected for illustration because it is presumbly the newest and least affected by leaching subsequent to the removal of the carbonates.

It is not intended to assert here that the actual section of limestone from which this soil came was constituted as here pictured. An infinite variety of arrangements could be assumed to produce the same result. The problem is indeterminate, from the data at hand.

Summary

The chief object of this paper is to draw attention to the peculiar distribution of the phosphate in these soil sections and to point out that it corresponds strikingly with the distribution of phosphate in the phosphatic limestone beds of this vicinity.

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STATEMENT

OF THE OWNERSHIP, MANAGEMENT, CIRCULATION, ETC., REQUIRED BY THE ACT OF CONGRESS OF AUGUST 24, 1912,

of Soil Science, published monthly at New Brunswick, N. J., for October 1, 1916.

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County of Middlesex,

Before me, a Notary Public in and for the State and county aforesaid, personally appeared Jacob G. Lipman, who, having been duly sworn according to law, deposes and says that he is the publisher of SOIL SCIENCE and that the following is, to the best of his knowledge and belief, a true statement of the ownership, management, etc., of the aforesaid publication for the date shown in the above caption, required by the Act of August 24, 1912, embodied in section 443, Postal Laws and Regulations, to wit:

1. That the names and addresses of the publisher, editor, managing editor, and business managers are:

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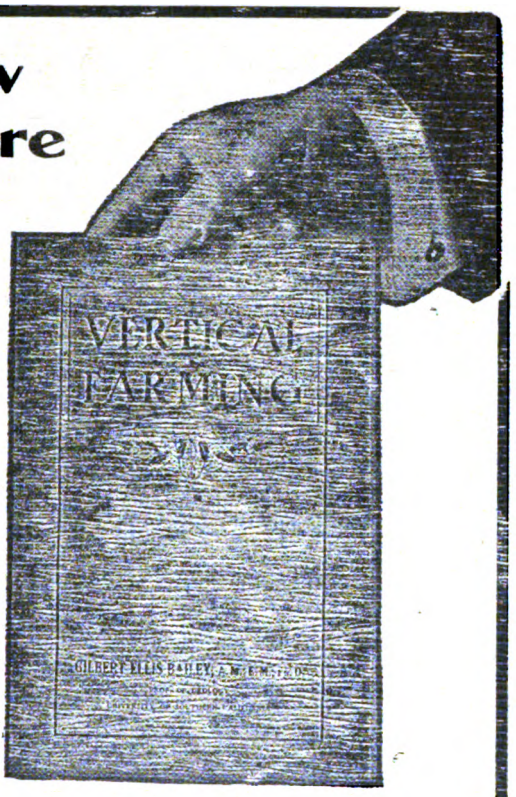

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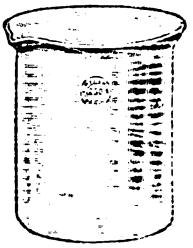

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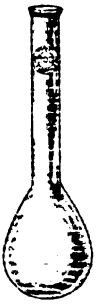

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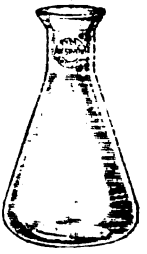

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SOIL SCIENCE

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SOIL SCIENCE

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VOL. II NEW BRUNSWICK, N. J., NOVEMBER, 1916 No. 5

THE ORGANIC MATTER OF THE SOIL: I. SOME DATA ON HUMUS, HUMUS CARBON AND HUMUS NITROGEN

BY

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INTRODUCTION

Since the time of Liebig, the nature of the organic matter of the soil has interested chemists, and every year sees extensive contributions to the voluminous literature. It would be far beyond the scope of the present paper to attempt a complete review of the literature and the bibliography will therefore be limited as much as possible to those papers which deal directly with the problems at hand.

Briefly the problems may be stated as follows:

1. Is the humus, the ammonia or alkaline hydroxide extract of soils, a typical soil product, formed in the soil by the action of bacteria and fungi, or can similar extracts be obtained from unchanged vegetable material?

2. Does the "humus," the "matière noire" of Grandeau, consist entirely of a black compound or compounds, or does it contain in addition a greater or less proportion of colorless substances whose presence is masked by a black pigment?

3. Does a 4 per cent sodium hydroxide solution extract the same substances from a soil as a 4 per cent ammonium hydroxide solution, and, if the same substances are extracted, is the same quantity extracted by each solvent?

4. Is the soil nitrogen present in a form different from that in which the nitrogen occurs in plant materials, or does the nitrogen of the soil exhibit the same solubilities as vegetable nitrogen?

[1] The work embodied in this paper was carried out while the author, now Associate Agricultural Bio-Chemist, was Associate Soil Chemist.

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In regard to the first and second questions: Hilgard's views on the nature of the ammonia extract of soils and his statements as to the normal processes of humification (17, p. 123-4) have been very widely accepted. He regards the humus of soils as a definite soil product, formed from vegetable material in the soil under the influence of fungous and bacterial growths: this conversion being most efficiently carried out in the presence of only a moderate amount of moisture, under the influence of a more or less rapid circulation of air, and in the presence of calcium carbonate to neutralize any acids which may be formed. Under these conditions the vegetable substance is converted into black, neutral, insoluble humus compounds.

Hilgard believed that the nitrogen contained in the "humified" portion of the soil organic matter was the only important part of the soil nitrogen which could be converted into nitrates under the influence of the nitrifying bacteria and that the nitrogen of plant debris must first pass through these intermediate stages before being available for the use of the higher plants. His views of the persistence of plant materials in soils are contained in the following statement:

"As a matter of course, the several organic compounds contained in plants may continue to exist in soils for some time, varying according to conditions of temperature and moisture. Thus dextrin, glucose, and even lecithin and nuclein have been reported to be found. The activity of the numerous fungous and bacterial ferments under favoring conditions, will, of course, limit the continued existence of such compounds somewhat narrowly, so that they can hardly be considered as active soil ingredients save in so far as they favor the development of the bacterial flora."

Grandeau (13) elaborated a method for the estimation of the "matière noire" of soils by first leaching the soil with dilute acid in order to set the humus free from its combination with the alkaline earths, removing the excess of acid by washing with water, then *moistening* the soil with ammonia and allowing it to stand for a short time (three to four hours, *cf.* Grandeau 1877, [14] p. 149), after which the humus solution was displaced by repeated washings with ammonical water. The *dark brown* solution so obtained was evaporated to dryness in platinum, weighed, ashed and the amount of "matière noire" and of ash recorded. Grandeau regarded the humus ash as an integral part of the humus.

Grandeau's method, more or less modified, is the basis for all modern methods for the estimation of humus, and Hilgard (17, p. 132) expressed a view, generally accepted in this country, of the nature of the substances dissolved, in the following statements:

"It has been usual to determine the amount of humus in soils by means of (dry or wet) combustion, calculating the humus from the

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carbonic dioxid so formed, while measuring the nitrogen gas directly. But in this process the entire organic matter of the soil, humified and unhumified, is indiscriminately included; and it is wholly uncertain to what extent the latter will ultimately become humus, from the nitrification of which plants are presumed chiefly to derive their nitrogen. In order to obtain definite results, the actual, functional humus must be extracted from the soil mass by some solvent which discriminates between the humified and unhumified organic matter. This cannot be done by direct extraction with caustic soda or potash, which inevitably dissolve unhumified matters and tend to expel ammonia from the humus; besides themselves acting as humifiers. *The only method now known which accomplishes this separation, practically excluding the unhumified while fully dissolving the humified matter is that of Grandeau.*"[1]

In view of the well known ability of ammonia solutions to dissolve organic compounds, it seemed highly improbable that ammonium hydroxide would dissolve only the "humified" portion. Experiments were therefore instituted in which *unleached* soils were extracted with 4 per cent ammonium hydroxide *and in every instance* a solution was obtained which was relatively colorless, but which, nevertheless, contained a very considerable amount of dissolved organic compounds. When this solution was evaporated to dryness on a water bath the compounds were more or less oxidized and a brown residue remained, in some respects resembling typical humus but lacking the black lustrous color. If the evaporation is carried out at 60° C. *in vacuo,* this browning can be largely prevented.

If one speculates on the nature of the soil organic matter, it becomes obvious that the variety of compounds which are present in a soil is limited only by those compounds which were present in the plants growing upon the soil, *plus* those compounds which compose the bodies of bacteria and protozoa, *plus* the compounds contained in the soil fungi, *plus* all of the various compounds which may be formed from the above sources by decay, oxidation, and all of the intricate chemical reactions which take place in converting dead organic material, either into living protoplasm, on the one hand, or into water, carbon dioxide and nitrogen on the other. Undoubtedly these organic compounds are not the product of "humification" but are derived from unchanged plant material, from protozoa, or from bacteria.

In certain experiments, unhumified vegetable materials yielded in some instances as much as 50 per cent of "humus," if by "humus" we mean the organic matter soluble in 4 per cent ammonia. In some of the experiments with unhumified vegetable materials the ammonia ex-

[1] Italics by the author.

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tract exhibited all of the properties of true "humus" solutions excepting that it lacked the true black color, being rather a very dark brown. It, therefore, appears extremely improbable that more than a small percentage of the humus, the "matière noire" extracted by Grandeau's method, is actually *black*. Recently the author has succeeded in isolating the black *soil pigment* and found that it is present in relatively small amounts.

In order to answer the third and fourth questions it was necessary to make a large series of experiments. These questions are important since the nitrogen content of "humus" has been determined by the method of Hilgard and Jaffa (18) in which sodium hydroxide replaces the ammonium hydroxide of the humus extraction. Alway and Bishop (1) recently called attention to the fact that "the correctness of the assumption that the ammonia solution dissolves the same compounds or the same proportion of total nitrogen as the alkaline hydroxides is open to serious question . . . A determination of the organic carbon in both solvents should be made, and if this is not the same, the nitrogen in the alkaline hydroxide solution is not to be regarded as that corresponding to the whole of the organic matter dissolved by the ammonia."

Such a study is reported in this paper, and it is found that not only is more carbon extracted by the alkaline hydroxide than by the ammonia, but that practically all of the nitrogen that is extractable from the soil after leaching with 1 per cent HCl is also extractable *without previous leaching*. Likewise none of the *soil pigment* is soluble in 4 per cent NaOH without previously leaching the soil, *and but very little is soluble in 4 per cent NaOH after leaching*, whereas, it is readily soluble in 4 per cent ammonia, after leaching with acid. In other words, 4 per cent sodium hydroxide does not extract the same compounds, nor the same proportion of compounds that are extracted by 4 per cent ammonium hydroxide, and the nitrogen figures obtained on sodium hydroxide extracts cannot be properly correlated with "humus" obtained by ammonia extraction.

Experimental

I. The Analytical Methods

The Preparation of the Soil Extracts and the Estimation of "Humus". Throughout this work only air-dry soils and vegetable materials were used. When one remembers the ease with which plant saps are decomposed by heat, it becomes exceedingly questionable whether the extracts of soils dried at 100° C. or 110° C. would contain the same compounds as those extracts made from unheated soils. Schreiner and Lathrop (26), Seaver and Clark (27, 28), Lyon and Bizzell (21), as well as numerous other investigators have made studies

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on heated soils and, almost without exception, the finding is that the solubility of the organic matter is greatly altered by heating.

Fifteen gm. of the air-dry soil or 5 gm. of the air-dry vegetable material, either with or without previous leaching with 1 per cent HCl as the case might be, were placed in a 1-liter bottle, 750 c.c. of 4 per cent NaOH, 2 per cent NaOH, or 4 per cent NH_4OH added, the bottle tightly stoppered with a rubber stopper and shaken continuously for one week in a shaking machine, in which the bottles were rotated "wheel fashion" so that *the soil was continually falling through the solution.* The speed was regulated so that the bottles made about ten revolutions per minute. At the end of the week the bottles were removed from the shaking machine and the soil was allowed to settle. In three or four days the soil had completely settled in those bottles to which sodium hydroxide had been added, and the clear supernatant liquid could be siphoned off for analysis.

In those bottles, however, to which ammonium hydroxide had been added, the soil did not settle readily. The following procedure was therefore adopted: Approximately 200 c.c. of the solution was siphoned off into a glass-stoppered cylinder and 3 gm. of K_2SO_4 added to precipitate the clay. After the clay had all settled out, leaving the supernatant liquid clear, portions of 50 c.c. were removed and used for the determination of organic carbon (see later). The remaining solution was siphoned off into a second glass-stoppered cylinder and 2 gm. of ammonium carbonate added to precipitate the clay as in Rather's (25) method. After all of the clay had precipitated, the solution was filtered and portions of from 150 to 250 c.c. were evaporated to dryness on a steam bath in weighed quartz dishes, then dried at 105° C., and ashed in an electric muffle. The loss on ignition was recorded as "humus" and the residue as "humus ash."

Undoubtedly in these determinations a higher humus content is shown than would be found by using any of the standard methods, such as Rather's (25), Hilgard's (16), the Moores-Hampton (23), etc., because of the greater length of time in contact with the ammonium hydroxide and because of the more thorough and constant shaking. However, *the results are all comparable with each other* and the carbon extracted by the ammonium hydroxide was extracted in exactly the same way as in the case of sodium hydroxide. It is impossible to "shake occasionally for 24 hours" as in the "official" (6) and Rather (25) methods and always have exactly the same conditions. Then again it was desired to approach as nearly as practicable to the maximum solubility of the organic matter. Had the bottles been shaken for two weeks instead of for one week, there would have been an increase in the amount of humus and carbon dissolved, but a longer time seemed impracticable.

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One noteworthy difference was observed between those soils which were treated in the unleached condition, and those previously leached with 1 per cent HCl: *i. e.*, the soil settled rapidly from the solution in the case of the leached soils plus NaOH but only slowly in the case of the unleached soil plus NaOH. The clay was floculated within a few minutes on adding ammonium carbonate to the ammonia extract of the leached soils, but it often happened that no floculation of the clay would take place for a week or more when ammonium carbonate was added to the extracts from the unleached soil. However, once the clay began to floculate in these extracts, it came down quite rapidly. This suggests a very different colloidial state of the clay in the two instances.

The Determination of Organic Carbon. Organic carbon was determined by wet combustion in the apparatus shown in figure 1. No novel features are claimed for this apparatus, since it is a combination of the best parts of several wet-combustion methods. However, in view of the accuracy of the results which can be obtained, it is thought worth while to describe the apparatus in some detail.

"A" contains a 1:1 solution of KOH in order to remove all CO_2 from the air aspirated through the apparatus. "E" contains a saturated solution of silver sulphate in 5 per cent H_2SO_4, in order to remove any traces of hydrochloric acid or chlorine which might be present. It is best to add a considerable excess of solid silver sulphate, allowing this to lie in the bottom of the U tube so as to have at all times a saturated solution. "F" is a Schmitz drying tube containing concentrated sulphuric acid. "G" contains anhydrous, granular, calcium chloride. "H" is the potash bulb filled with 1:1 KOH solution, and "I" contains soda lime, to protect the potash bulb from moisture and carbon dioxide from the outside air.

Truog (30), recently criticized the weighing of CO_2 in a potash bulb because of the error which might be introduced, due to the small increase in weight in a relatively heavy piece of apparatus, and also since the drying materials are not the same at each end of the potash bulb. The first criticism can be obviated by always weighing the potash bulb against a similar bulb used as a counterpoise. In this manner barometric and temperature changes are compensated. All weights were made with the use of a second potash bulb for a counterpoise, the weight of the counterpoise being within 2 or 3 gm. of the potash bulb used.

The second criticism has been met by fastening a small tube filled with anhydrous calcium chloride upon the intake tube of the "Gomberg" and weighing this as an integral part of the potash bulb. In this manner the solution of KOH is protected at both outlets from evaporation. By these modifications the weighing of CO_2 becomes as accurate as, or more accurate than, any titrametric method.

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"D" is an electric furnace containing a copper oxide spiral which is maintained at a dull red heat during the determination, as well as a silver spiral near the exit end of the furnace tube. This is thought necessary in order to burn any carbon monoxide or volatile organic compounds which may be formed by the chromic acid oxidation. The furnace can be easily constructed in the laboratory. It consists of a silica combustion tube 15 inches long with an inner diameter of 5/8 of an inch, glazed inside and out. Around the middle of this tube is wound approximately 50 feet of No. 27 "nichrom" wire, which had been previously coiled into a spiral of about 5 mm. diameter. The nichrom winding should cover about 6 inches of the silica tube. The windings

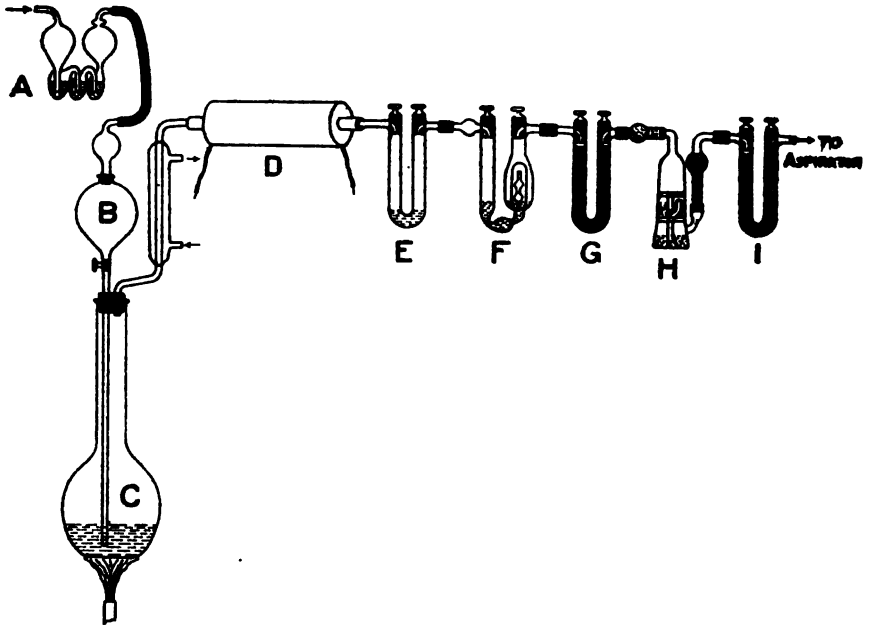

Fig. 1.—Apparatus for the determination of organic carbon by wet combustion.

are embedded in "alundum" cement, or in a cement made from equal parts of powdered moulding clay and finely powdered quartz. After drying at 110° C. until all moisture is removed, the cement is hardened by heating the coil by means of an electric current. A very convenient outer jacket for the furnace can be made from a porous clay cup, 3 inches in diameter by 7 inches high, such as is used in wet electric batteries. The space between the cement covering the wire, and the wall of the porous cup is tightly packed with loose asbestos fiber and the open end of the cup closed with a piece of asbestos board.

A furnace of these dimensions can be operated on a 220-volt circuit in series with a rheostat made from 110-volt electric light bulbs. The

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rheostat for my own furnace consists of four 25-watt tungsten, and one 55-watt carbon bulbs, connected in parallel. The furnace heats to dull red in about 15 minutes when all lights are burning, and is kept at that temperature indefinitely by burning the carbon and two of the tungsten lamps, or a current consumption of 105 watts. This furnace has been run practically 8 hours a day for a year and a half and shows no sign of deterioration.

A carbon determination is made as follows: 50 c.c. of soil extract (or 1 to 2 gm. of soil plus 50 c.c. CO_2-free water) is placed in the 200-c.c. long-necked Kjeldahl flask "C," 5 gm. of potassium dichromate is added and the flask connected with the condenser and separatory funnel; 75 c.c. of concentrated H_2SO_4 is then slowly run in from the separatory funnel "B", in the top of which is a ground glass connection to which can be fastened the rubber tube leading to the potash guard bulb "A". A rubber stopper cannot be used in the top of the separatory funnel because of the danger of dissolving some rubber in the sulphuric acid.

After all of the sulphuric acid has been run in, the contents of the flask are mixed by gentle shaking and the aspiration is started. It has been found advisable to aspirate about 2½ liters of air through the apparatus, the entire time of aspiration being about 2½ hours. During the first two-thirds of the aspiration the contents of the flask are kept just below the boiling temperature by means of a low flame. The flask cools during the last one-third of the aspiration so that a second determination can be started at once.

By this method we obtain total carbon, *i. e.*, both organic carbon and inorganic carbonates, and the organic carbon is obtained by making a separate determination of the carbonate CO_2 and substracting this from the total CO_2.

After some work had been done it was found that it was impossible accurately to determine carbon in a 4 per cent solution of ammonium hydroxide without previously evaporating off the ammonia. Ames and Gaither (5) report the analysis of 4 per cent ammonia extracts of soils by chromic oxidation and obtain an average of 1.807 per cent carbon for the extracts evaporated to dryness and 1.970 per cent carbon for the extracts not evaporated. They suggest that this difference "can be accounted for by the loss of volatile and easily oxidizable carbonaceous matter through evaporation on the steam bath." If this were the case, one would think that a similar, and greater loss would be observed when soils are dried at 110° C., but such is not the case. When 50 c.c. of 4 per cent ammonia is oxidized by chromic acid the potash bulb may increase as much as 0.0260 gm., whereas when this same solution is evaporated to dryness in the Kjeldahl flask *in vacuo* at 60° C., there is a "blank" not greater than 0.0010 gm. CO_2. The factors involved in this

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behavior have not been ascertained but it is possible that ammonium cyanide or some of the aliphatic amines are present. Nitric acid, and oxides of nitrogen are formed to some extent but they are not important factors since the KOH of the potash bulb gave only a faint trace of nitrates with diphenylamine after 8 or 10 oxidations of 50 c.c. of 4 per cent ammonia.

In all of the following work the ammonia solutions were evaporated to dryness *in vacuo* at 60° C. in the Kjeldahl flask in which they were subsequently oxidized, 50 c.c. distilled water, free from CO_2, added and treated with chromic acid as above.

One should here present some data showing the accuracy of the wet-combustion method. A few determinations of organic carbon in soils are shown in Table I. It is necessary, however, to reserve the really conclusive proof for the second paper in this series. It suffices to say, however, that *organic carbon was determined on the original soil, then on sixteen consecutive soil extracts and, lastly, on the residual soil, and a recovery of* 101.8 *per cent of the original carbon was effected.* The 1.8 per cent increase represented an error of only 0.00032 gm. carbon per determination.

TABLE I

THE AGREEMENT BETWEEN ORGANIC CARBON DETERMINATIONS IN SOILS OXIDIZED BY WET COMBUSTION AND BY IGNITION WITH COPPER OXIDE IN OXYGEN

Soil	Per cent Organic Carbon by Wet Combustion			Per cent Organic Carbon by Copper Oxide in Oxygen
	I	II	Average	
Carrington Silt Loam ..	4.757	4.709	4.733	4.701
Hempstead Silt Loam...	3.379	3.367	3.373	3.324
Fargo Silt Loam.....	9.959	10.080	10.020	10.030
Sphagnum-covered Peat..	49.290	49.340	49.320	49.010
Forest-covered Loess.....	1.637	1.639	1.638	1.612

The Determination of Inorganic Carbon. The determination of CO_2 derived from carbonates is carried out in an apparatus exactly like that used for total carbon, with the exception that the electric furnace is omitted.

The soil extract is placed in the Kjeldahl flask and 10 c.c of 85 per cent phosphoric acid added through the separatory funnel. Aspiration is started and the contents of the flask heated to about 80° C. to ensure complete action, and then allowed to cool. In the case of soils from 5 to 10 gm. of soil are taken, 50 c.c. of CO_2-free water added, and the carbonates decomposed with 25 c.c. of 5 per cent (by weight) HCl. Otherwise the conditions are the same as for the soil solutions.

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The Determination of Nitrogen. Nitrogen was determined on the soils and soil extracts in the usual manner, 25 to 35 c.c. H_2SO_4, 10 gm. K_2SO_4 and a crystal of $CuSO_4$ being used. All titrations were made with N/14 acid and alkali so that the figures obtained represented milligrams of nitrogen without necessitating a calculation.

II. The Samples Analysed

Seventeen samples of soil or vegetable materials were analysed. The soils used were selected from those in stock in the laboratory and recently collected in connection with other investigations. All were from Minnesota with the exception of No. 6 which was from California and No. 8 from Nebraska. The soils are named in accordance with the surveys of the Bureau of Soils of the U. S. Department of Agriculture when such surveys are available.

1. *Fargo Clay Loam.* The sample analysed consisted of a composite made from 144 borings taken to a depth of 8 inches from a 20-acre field on the Northwest Sub-station farm, Crookston, Minn. The sample was highly calcareous. This soil type has been described by Mangum and Schroeder (22). Fifteen gm. of air-dry soil containing 3.92 per cent of moisture were used in all the extractions.

2. *Fargo Silt Loam.* This sample was a composite made from 100 borings to a depth of 6 inches, 20 borings being taken from each of 5 virgin fields near Nerstrand, Rice Co., Minn. The sample had a neutral reaction. The soil type has been described by Burke and Kolbe (10). The air-dry soil contained 14.89 per cent of moisture. Fifteen gm. portions were used for the preparation of the extracts.

3. *Carrington Silt Loam.* This soil sample was likewise a composite of 100 borings to a depth of 6 inches, 20 borings being taken from each of 5 virgin fields near Nerstrand, Rice Co., Minnesota. The sample was strongly acid. The soil type is described by Burke and Kolbe (10). Fifteen gm. of air-dry soil were used in preparing the extracts, the moisture content being 6.22 per cent.

4. The *Prairie-covered Loess* sample consisted of a composite from 50 borings taken to a depth of 1 foot, 10 borings being taken from each of 5 virgin fields near Luverne, Rock Co., Minn. The sample was somewhat calcareous. Fifteen-gm. portions of air-dry soil were taken for analysis, the moisture content being 7.89 per cent.

5. The *Forest-covered Loess* sample was taken from 5 virgin fields near Caledonia, Huston Co., Minn. Ten borings to a depth of 1 foot were taken from each field and equal weights from each boring combined in the composite sample. The sample was strongly acid. Fifteen gm. of air-dry soil were used in each experiment, the moisture content being 1.87 per cent.

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6. The *"Berkeley Adobe"*. It seemed desirable to include in these experiments a sample of a truly arid soil. For this I used a composite of two samples from Berkeley, California, collected by Professor Alway, and by him kindly placed at my disposal. They were the soils "A" and "B" on which some analyses have been reported by Alway and Bishop (1). They give the history of the samples as follows:

"Samples A and B were both taken from the high hill just east of the buildings on the grounds of the University of California. Sample A is a composite of 20 samples from near the summit, and B of the same number from the lighter colored soil to the west, below the summit." The samples were taken to a depth of 3 inches from virgin soil. In order to obtain a sufficiently large sample it was necessary to combine all that remained of the two samples which differed considerably in weight. Unfortunately the samples were not weighed before combining.

The soil analysed was slightly acid and contained 2.75 per cent of moisture. Fifteen-gm. portions were used in preparing the extracts.

7. *Hempstead Silt Loam.* A composite sample from 36 borings to a depth of 6 inches was taken from 12 plots on the Minnesota Agricultural Experiment Station farm, St. Paul. No commercial fertilizer had been applied, but the land had long been under cultivation. The soil type has been described by Smith and Kirk (29). The sample was strongly acid. Fifteen-gm. portions of air-dry soil containing 3.07 per cent of moisture were used in the different experiments.

8. *Marshall Silt Loam.* This soil was part of a bulk sample taken to a depth of 6 inches from a long cultivated (40 years) field on the farm of the Nebraska Agricultural Experiment Station at Lincoln. The soil type has been described by Burgess and Worthen, (9). The sample was slightly acid, and contained 2.83 per cent of moisture. Fifteen-gm. portions were used in the experiments.

9. A sample of strongly *acid "muck" soil* was obtained from a bog about 2 miles from the farm of the Minnesota Agricultural Experiment Station, St. Paul. The sample is a composite of 10 samples taken to a depth of 8 inches, lengthwise of the bog. The vegetation of the bog was largely "cat-tails" and rushes. Portions of air-dry soil weighing 7.5 gm. each, containing 5.60 per cent of moisture were used in the extractions.

10. *Sphagnum-covered peat.* This sample, which was very strongly acid, was prepared from a large bulk sample taken from a sphagnum-covered bog on the experimental farm near Grand Rapids, Minn. The superficial layer of sphagnum and shrubs was first removed and a sample of the underlying peat taken to a depth of 8 inches. The peat was poorly decomposed. The sample was prepared by grinding the material

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to a powder in a ball mill. Five-gm. portions, containing 5.90 per cent of moisture were used in the extracts. An analysis of the peat is shown in Table II.

11. A sample of *Calcareous Black Peat* was selected from a large bulk sample taken to a depth of 8 inches from a grass bog near the Minnesota Agricultural Experiment Station farm, St. Paul. The peat was black and well decomposed. The peat was ground to a powder in a ball mill before using. An analysis of the peat is shown in Table II. For the extractions, 5 gm. of material containing 6.40 per cent of moisture were used.

TABLE II

COMPARATIVE "PEAT ANALYSES" ON THE PEATS AND VEGETABLE MATERIALS USED[1]

	Per cent Volatile	Per cent Ash	Per cent Insoluble	Per cent CaO	Per cent P_2O_5	Per cent N	Per cent C
"Muck"	26.75	73.25	67.97	0.28	0.11	1.34	14.58
Sphagnum-covered Peat	90.59	9.41	6.12	0.63	0.24	2.00	49.32
Black Peat	78.68	21.32	10.26	4.63	0.47	2.94	42.81
Brown Peat	86.10	13.90	11.37	1.22	0.33	2.82	46.34
Grass from Brown Peat Bog	92.97	7.03	3.08	0.51	0.24	1.16	47.02
Oat Plants	86.29	13.71	6.15	0.49	1.33	2.13	42.48
Alfalfa Hay	88.47	11.53	1.84	2.47	0.65	3.80	44.26
Sweet Fern Leaves	92.44	7.56	4.88	0.92	0.17	1.59	48.34
Oak Leaves	94.13	5.87	0.66	2.26	0.27	1.00	48.63

[1] The analyses were made according to the method of the Bremen Peat Experiment Station (20), with the exception that all calcium determinations but that of the "Muck" soil were made by the method recently proposed by the author (12). Carbon was determined by wet combustion.

12. A sample of *acid Brown Peat* was selected from a large bulk sample taken from the first 8 inches of a grass bog about a half mile from the buildings of the Minnesota Agricultural Experiment Station. The peat was poorly decomposed, the sample utilized being ground to a powder in a ball mill. An analysis of the peat is shown in Table II. Five-gm. portions containing 11.31 per cent of moisture, were taken for the extraction experiments.

13. A sample of the *grass growing on the Brown Peat Bog* was secured in September, ground to a powder in a ball mill, and used for analysis. This sample, tested by methods for "soil acidity," showed a strongly acid reaction. Five-gm. portions containing 8.50 per cent of moisture were used in the extractions. A "peat analysis" on the grass is shown in Table II.

14-17. A sample of *ripe oat plants* (straw and grain, without the roots) was ground to a powder in the ball mill and used for comparative analyses. Likewise samples of *alfalfa hay, sweet fern leaves* (dry and ready to fall) and *oak leaves (Quercus coccinea,* Muench) which

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had remained on the tree until March, were secured and prepared as above. "Peat analyses" are shown in Table II. All samples showed strong acidity. Five-gm. portions of each were used for the extractions. The moisture percentages were, oats, 6.40; alfalfa, 6.00; sweet fern, 1.35; and oak leaves 1.95.

III. The Presentation of the Analytical Data

The general plan of the experiments. A series of symbols have been used to designate the treatment to which the soil has been subjected in order to obtain the solution in question. The following symbols will be used throughout this paper: "A" signifying *acid* and "B" signifying *base.*

A(HCl) is the 1 per cent hydrochloric extract of the soil, obtained by leaching the soil on a filter with 1 per cent HCl until no more calcium is removed and then washing with water to the absence of chlorides. This solution was utilized only for the determination of nitrogen.

AB(NH_4OH) is the ordinary black humus solution obtained by first leaching the soil with 1 per cent HCl and subsequent extraction of the humus with 4 per cent NH_4OH solution by the method above described.

AB(NaOH) is obtained exactly as AB(NH_4OH) with the exception that the ammonia solution is replaced by 4 per cent NaOH solution. This solution is not black, but usually an intense brown.

B(NH_4OH) is the 4 per cent ammonia extract obtained without previous leaching of the soil with 1 per cent HCl. This solution is usually only slightly colored, the color varying from very light straw to light brown.

B(NaOH) is similar to B(NH_4OH) with the ammonia replaced by 4 per cent NaOH solution. This solution is usually darker in color than B(NH_4OH) but never becomes *black,* being usually more or less brown.

BA(HCl) is the solution obtained by leaching the residue from B(NaOH) with 1 per cent HCl to the absence of calcium, and further washing with water to the absence of chlorides.

BAB(NaOH) is the extract obtained from the leached soil residue from BA(HCl) when this is shaken for one week with 4 per cent NaOH. This solution is usually darker than B(NaOH) or BA(NaOH) but is always brown, never black.

BAB + H_2O The soil residue from BAB(NaOH) is drained on a hardened filter until practically all of solution BAB(NaOH) has been removed. The soil residue, still alkaline from some adhering sodium hydroxide, is added to 750 c.c. of distilled water and the mixture shaken for one week. The solution obtained is designated BAB + H_2O. This solution, with soils, is always *jet black,* when in volume and contains the

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true *soil pigment* which can be precipitated from this solution by the addition of NaOH equal to 4 per cent concentration, or by acidification.

In practically all of the experiments, the soil extracts were prepared in duplicate so that in the following tables the total carbon percentages represent the average of two closely agreeing duplicate determinations, the total nitrogen percentages represent the average of three or more closely agreeing duplicate determinations, the humus and humus ash percentages represent two closely agreeing determinations from separate soil extracts, the carbon percentages of the solutions are likewise averages of closely agreeing duplicate determinations from two distinct soil extracts, while the nitrogen percentages are in most cases the average of four determinations, duplicate analyses being made on from 200 to 250 c.c. of solution from each soil extract.

The individual determinations are not recorded in detail because of the excessive amount of space which would be necessary. All percentages are calculated to the basis of oven-dry soil.

Tables III to XVIII give the analytical data obtained. Table XIX shows a summary of these data. Table X varies somewhat from the usual procedure in that the unleached soil was extracted with 4 per cent NaOH *twice* before leaching with acid for BAB (NaOH). It was necessary to present these analyses in order to give some idea of the amount of nitrogen retained in the soil residue. But in the graphs and in the average tables data are taken from another series of analyses made in the usual manner on the same soil (Marshall Silt Loam), so that all averages are correct.

Table XX shows the approximate colors of the solutions obtained. The colors were approximated by placing a portion of the solution in flat porcelain evaporating dishes so that the depth of the liquid was 1 cm. The color of the center of the solution was then matched against the standard color chart facing page 2334 of Funk and Wagnall's (11) "New Standard Dictionary".

Quantitative measurements of the intensity of coloration are given in Tables XXI and XXII. Table XXI shows the *relative coloration within each series* as referred to the ordinary humus solution AB (NH_4OH). Table XXII gives the quantitative relationships determined colorimetrically and photometrically (4), of the standards used in Table XXI, the standard in this instance being a 0.0063 per cent solution of the "soil pigment".[1]

In this table is also shown, for comparative purposes, the weight of "humus" actually obtained per unit volume of solution, as well as the calculated "indicated approximate" and "actual" percentages of soil pigment in the humus solution, AB(NH_4OH).

[1] The description of the isolation and properties of this "soil pigment" will comprise the second paper of this series.

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TABLE III

A SUMMARY OF THE DATA ON FARGO CLAY LOAM

Total N in Soil	0.250%	C/N
Total Organic C in Soil	2.678%	
Carbonate CO_2	2.360%	10.72

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Soil	Per cent of Total N	Per cent of Soil	Per cent of Total C				
A (HCl)	0.007	2.80	.....	.....	.....	.....	.....	.....
AB (NaOH)	0.153	61.20	1.537	57.40	.....	.....	10.05	.....
AB (NH_4OH)	.....	.....	1.423	53.14	2.66	0.56	.....	53.50
B (NH_4OH)	.....	.....	0.476	17.77	0.95	0.46	.....	50.10
B (NaOH)	0.127	50.81	0.979	36.56	.....	.....	7.71	.....
BA (HCl)	.....	.....	.....	.....	.....	.....	.....	.....
BAB (NaOH)	0.070	28.00	0.531	19.83	.....	.....	7.59	.....
BAB + H_2O	0.028	11.20	0.428	15.98	.....	.....	15.29	.....
Soil Residue	0.034	13.60	*0.740	*27.63	.....	.....	21.76	.....
Total	0.259	103.61	.....	*100.00	.....	.....	.....	.....

* By difference.

TABLE IV

A SUMMARY OF THE DATA ON FARGO SILT LOAM

Total N in Soil	0.823%	C/N
Total Organic C in Soil	10.020%	
Carbonate CO_2	0.200%	12.17

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Soil	Per cent of Total N	Per cent of Soil	Per cent of Total C				
A (HCl)	0.021	2.55	.....	.....	.....	.....	.....	.....
AB (NaOH)	0.613	74.49	5.990	59.78	.....	.....	9.77	.....
AB (NH_4OH)	.....	.....	5.174	51.64	9.91	0.72	.....	52.20
B (NH_4OH)	.....	.....	1.774	17.71	3.65	0.46	.....	48.62
B (NaOH)	0.584	70.96	4.555	45.46	.....	.....	7.80	.....
BA (HCl)	.....	.....	.....	.....	.....	.....	.....	.....
BAB (NaOH)	0.090	10.93	1.647	16.44	.....	.....	18.30	.....
BAB + H_2O	0.080	9.72	1.530	15.27	.....	.....	19.13	.....
Soil Residue	0.087	10.57	*2.288	*22.83	.....	.....	26.29	.....
Total	0.841	102.18	.....	*100.00	.....	.....	.....	.....

* By difference.

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TABLE V

A SUMMARY OF THE DATA ON CARRINGTON SILT LOAM

Total N in Soil	0.371%	C/N
Total Organic C in Soil	4.733%	
Carbonate CO_2	0.090%	12.76

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Soil	Per cent of Total N	Per cent of Soil	Per cent of Total C				
A (HCl)	0.009	2.45	.....	.....	.....	.....	.....	.....
AB (NaOH)	0.266	71.69	2.890	61.05	.....	.....	10.86	.....
AB (NH_4OH)	.....	.....	2.525	53.34	4.95	0.40	.....	51.01
B (NH_4OH)	.....	.....	1.436	30.35	2.86	0.34	.....	50.20
B (NaOH)	0.271	73.05	2.349	49.63	.....	.....	8.67	.....
BA (HCl)	.....	.....	.....	.....	.....	.....	.....	.....
BAB (NaOH)	0.030	8.17	0.653	13.79	.....	.....	21.77	.....
BAB + H_2O	0.032	8.71	0.737	15.57	.....	.....	23.03	.....
Soil Residue	0.044	11.99	*0.994	*21.00	.....	.....	22.59	.....
Total	0.383	101.92	.....	*100.00	.....	.....	.....	.....

* By difference.

TABLE VI

A SUMMARY OF THE DATA ON PRAIRIE-COVERED LOESS

Total N in Soil	0.301%	C/N
Total Organic C in Soil	3.704%	
Carbonate CO_2	0.240%	12.30

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Soil	Per cent of Total N	Per cent of Soil	Per cent of Total C				
A (HCl)	0.010	3.32	.....	.....	.....	.....	.....	.....
AB (NaOH)	0.194	64.46	2.049	55.31	.....	.....	10.56	.....
AB (NH_4OH)	.....	.....	1.737	46.88	3.40	0.42	.....	51.08
B (NH_4OH)	.....	.....	0.702	18.95	1.42	0.33	.....	49.43
B (NaOH)	0.186	61.79	1.548	41.78	.....	.....	8.32	.....
BA (HCl)	.....	.....	.....	.....	.....	.....	.....	.....
BAB (NaOH)	0.049	16.28	0.798	21.53	.....	.....	16.29	.....
BAB + H_2O	0.025	8.20	0.500	13.50	.....	.....	20.00	.....
Soil Residue	0.060	19.94	*0.086	*23.16	.....	.....	14.30	.....
Total	0.320	106.21	.....	*100.00	.....	.....	.....	.....

* By difference.

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TABLE VII

A SUMMARY OF THE DATA ON FOREST-COVERED LOESS

Total N in Soil	0.128%	C/N	
Total Organic C in Soil	1.638%		
Carbonate CO	0.120%	12.79	

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Soil	Per cent of Total N	Per cent of Soil	Per cent of Total C				
A (HCl)	0.0040	3.12	.....	.....	.....	.....	.....	.....
AB (NaOH)	0.0920	71.88	1.018	62.13	.....	.....	11.06	.....
AB (NH_4OH)	.....	.....	0.821	50.12	1.795	0.45	.....	45.73
B (NH_4OH)	.....	.....	0.570	34.80	1.160	0.25	.....	49.14
B (NaOH)	0.0840	65.49	0.796	48.60	.....	.....	9.50	.....
BA (HCl)	.....	.....	.....	.....	.....	.....	.....	.....
BAB (NaOH)	0.0140	10.93	0.310	18.92	.....	.....	22.15	.....
BAB + H_2O	0.0075	5.86	0.234	14.29	.....	.....	31.20	.....
Soil Residue	0.0235	18.36	*0.298	*18.20	.....	.....	12.68	.....
Total	0.1290	100.64	.....	*100.00	.....	.....	.....	.....

* By difference.

TABLE VIII

A SUMMARY OF THE DATA ON BERKELEY ADOBE

Total N in Soil	0.239%	C/N	
Total Organic C in Soil	2.919%		
Carbonate CO_3	0.230%	12.21	

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Soil	Per cent of Total N	Per cent of Soil	Per cent of Total C				
A (HCl)	0.010	4.18	.....	.....	.....	.....	.....	.....
AB (NaOH)	0.159	66.53	1.654	56.67	.....	.....	10.40	.....
AB (NH_4OH)	.....	.....	1.260	43.17	2.39	0.48	.....	52.72
B (NH_4OH)	.....	.....	0.658	22.54	1.24	0.33	.....	53.07
B (NaOH)	0.157	65.69	1.385	47.45	.....	.....	8.82	.....
BA (HCl)	0.007	2.93	.....	.....	.....	.....	.....	.....
BAB (NaOH)	0.026	10.87	0.505	17.30	.....	.....	19.42	.....
BAB + H_2O	0.013	5.44	0.305	10.45	.....	.....	23.46	.....
Soil Residue	0.034	14.22	*0.724	*24.80	.....	.....	21.29	.....
Total	0.237	99.15	.....	*100.00	.....	.....	.....	.....

* By difference.

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TABLE IX

A SUMMARY OF THE DATA ON HEMPSTEAD SILT LOAM

Total N in Soil	0.256%	C/N
Total Organic C in Soil	3.373%	
Carbonate CO_2	0.020%	13.17

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Soil	Per cent of Total N	Per cent of Soil	Per cent of Total C				
A (HCl)	0.008	3.12	.....	.....	.....	.....	.....	.....
AB (NaOH)	0.186	72.66	1.936	57.40	.....	.....	10.41	.....
AB (NH_4OH)	.....	.....	1.865	55.30	3.61	0.52	.....	51.66
B (NH_4OH)	.....	.....	0.929	27.54	2.00	0.62	.....	46.45
B (NaOH)	0.168	65.63	1.530	45.36	.....	.....	9.10	.....
BA (HCl)	.....	.....	.....	.....	.....	.....	.....	.....
BAB (NaOH)	0.039	15.24	0.730	21.64	.....	.....	18.72	.....
BAB + H_2O	0.031	12.12	0.724	21.47	.....	.....	23.35	.....
Soil Residue	0.033	12.89	*0.389	*11.53	.....	.....	11.79	.....
Total	0.271	105.88	.....	*100.00	.....	.....	.....	.....

* By difference.

TABLE X

A SUMMARY OF THE DATA ON MARSHALL SILT LOAM

Total N in Soil	0.237%	C/N
Total Organic C in Soil	2.670%	
Carbonate CO_2	0.220%	11.27

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Soil	Per cent of Total N	Per cent of Soil	Per cent of Total C				
A (HCl)	0.009	3.80	.....	.....	.....	.....	.....	.....
AB (NaOH)	0.153	65.31	1.560	58.42	.....	.....	10.20	.....
AB (NH_4OH)	.....	.....	1.510	56.56	2.85	0.37	.....	52.99
B (NH_4OH)	.....	.....	0.791	29.63	1.49	0.39	.....	53.09
B (NaOH)	0.152	64.13	1.270	47.56	.....	.....	8.36	.....
B + B (NaOH)	0.048	20.25	0.470	17.60	.....	.....	9.79	.....
(B + B) AB	0.019	8.02	0.377	14.12	.....	.....	19.84	.....
(B + B) AB + H_2O	0.017	7.17	0.426	15.95	.....	.....	25.06	.....
Soil Residue	0.025	10.55	*0.127	*4.76	.....	.....	5.08	.....
Total	0.261	110.12	.....	*100.00	.....	.....	.....	.....

* By difference.

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TABLE XI

A SUMMARY OF THE DATA ON AN ACID "MUCK" SOIL

Total N in Soil	1.340%	C/N
Total Organic C in Soil	14.580%	
Carbonate CO_3	None	10.88

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Soil	Per cent of Total N	Per cent of Soil	Per cent of Total C				
A (HCl)	0.034	2.54	.....	.....	.....	.....	.....	.....
AB (NaOH)	0.818	61.05	6.308	43.25	.....	.....	7.71	.....
AB (NH_4OH)	.....	.....	3.715	25.48	7.14	0.30	.....	52.04
B (NH_4OH)	.....	.....	2.814	19.30	5.82	†1.88	.....	48.35
B (NaOH)	0.778	58.06	5.374	36.85	.....	.....	6.91	.....
BA (HCl)	.....	.....	.....	.....	.....	.....	.....	.....
BAB (NaOH)	0.151	11.27	1.798	12.33	.....	.....	11.91	.....
BAB + H_2O	0.089	6.64	1.415	9.70	.....	.....	15.90	.....
Soil Residue	0.303	22.61	*5.993	*41.10	.....	.....	19.78	.....
Total	1.321	98.58	.....	*100.00	.....	.....	.....	.....

* By difference.

† The water from this muck bog carried 0.2220 gm. of ash per liter. This ash was largely calcium sulphate.

TABLE XII

A SUMMARY OF THE DATA ON AN ACID SPHAGNUM-COVERED PEAT

Total N in Peat	2.00%	C/N
Total Organic C in Peat	49.32%	
Carbonate CO_3	None	24.66

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Peat	Per cent of Total N	Per cent of Peat	Per cent of Total C				
A (HCl)	0.150	7.50	.....	.....	.....	.....	.....	.....
AB (NaOH)	1.468	73.41	26.20	53.13	.....	.....	17.84	.....
AB (NH_4OH)	.....	.....	16.96	34.40	32.91	1.47	.....	51.54
B (NH_4OH)	.....	.....	17.78	36.06	34.40	1.79	.....	51.69
B (NaOH)	1.697	84.86	27.40	55.56	.....	.....	16.14	.....
BA (HCl)	.....	.....	.....	.....	.....	.....	.....	.....
BAB (NaOH)	0.145	7.25	4.425	8.97	.....	.....	30.52	.....
BAB + H_2O	0.066	3.30	2.06	4.18	.....	.....	31.22	.....
Soil Residue	0.206	10.30	*15.43	*31.28	.....	.....	74.89	.....
Total	2.114	105.71	.....	*100.00	.....	.....	.....	.....

* By difference.

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TABLE XIII

A SUMMARY OF THE DATA ON A CALCAREOUS BLACK GRASS-PEAT

Total N in Peat	2.94%	C/N	
Total Organic C in Peat	42.81%		
Carbonate CO_2	0.60%	14.56	

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Peat	Per cent of Total N	Per cent of Peat	Per cent of Total C				
A (HCl)	0.081	2.76	.....	.....	.....	.....	.....	.....
AB (NaOH)	2.350	79.95	27.04	63.17	.....	.....	11.50	.....
AB (NH_4OH)	.....	.....	14.70	34.34	28.71	1.74	.....	51.19
B (NH_4OH)	.....	.....	6.51	15.21	12.97	1.13	.....	50.19
B (NaOH)	2.146	73.00	22.68	52.98	.....	.....	10.57	.....
BA (HCl)	.....	.....	.....	.....	.....	.....	.....	.....
BAB (NaOH)	0.390	13.26	7.42	17.34	.....	.....	19.02	.....
BAB + H_2O	0.186	6.33	4.30	10.05	.....	.....	23.12	.....
Soil Residue	0.119	4.05	*8.41	*19.64	.....	.....	70.68	.....
Total	2.841	96.64	.....	*100.00	.....	.....	.....	.....

* By difference.

TABLE XIV

A SUMMARY OF THE DATA ON AN ACID BROWN GRASS-PEAT

Total N in Peat	2.818%	C/N	
Total Organic C in Peat	46.34%		
Carbonate CO_2	None	16.44	

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Peat	Per cent of Total N	Per cent of Peat	Per cent of Total C				
A (HCl)	0.066	2.34	.....	.....	.....	.....	.....	.....
AB (NaOH)	2.176	77.23	28.08	60.59	.....	.....	12.90	.....
AB (NH_4OH)	.....	.....	20.14	43.45	39.22	1.87	.....	51.35
B (NH_4OH)	.....	.....	17.36	37.45	35.29	2.25	.....	49.19
B (NaOH)	2.143	76.05	27.19	58.66	.....	.....	12.69	.....
BA (HCl)	0.041	1.46	.....	.....	.....	.....	.....	.....
BAB (NaOH)	0.281	9.97	4.88	10.53	.....	.....	17.37	.....
BAB + H_2O	0.136	4.83	2.52	5.44	.....	.....	18.56	.....
Soil Residue	0.202	7.17	*11.75	*25.36	.....	.....	58.17	.....
Total	2.803	99.48	.....	*100.00	.....	.....	.....	.....

* By difference.

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TABLE XV

A SUMMARY OF THE DATA ON GRASS CUT FROM THE ACID BROWN PEAT BOG

Total N in Material	1.164%	C/N
Total Organic C in Material	47.020%	40.39

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Material	Per cent of Total N	Per cent of Material	Per cent of Total C				
A (HCl)	0.149	12.80	.....	.....	.....	.....	.....	.....
AB (NaOH)	0.948	81.43	26.900	57.22*	.....	.....	28.38	.....
AB (NH_4OH)	.....	.....	16.530	35.16	31.95	2.44	.....	51.74
B (NH_4OH)	.....	.....	18.300	38.92	36.46	†4.45	.....	50.20
B (NaOH)	1.041	89.41	30.150	64.12	.....	.....	28.97	.....
BA (HCl)	0.033	2.83	.....	.....	.....	.....	.....	.....
BAB (NaOH)	0.037	3.18	2.370	5.04	.....	.....	64.00	.....
BAB + H_2O	None	None	0.451	0.96	.....	.....	.....	.....
Soil Residue	0.025	2.15	*14.050	*29.87	.....	.....	561.00	.....
Total	1.136	97.57	.....	*100.00	.....	.....	.....	.....

* By difference.

† Probably largely inorganic plant constituents.

TABLE XVI

A SUMMARY OF THE DATA ON THE ENTIRE OAT PLANT

Total N in Material	2.131%	C/N
Total Organic C in Material	42.480%	19.48

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Material	Per cent of Total N	Per cent of Material	Per cent of Total C				
A (HCl)	0.737	34.58	.....	.....	.....	.....	.....	.....
AB (NaOH)	1.238	58.09	20.330	47.85	.....	.....	16.43	.....
AB (NH_4OH)	.....	.....	14.400	33.90	26.65	2.63	.....	54.04
B (NH_4OH)	.....	.....	17.150	40.36	36.09	†4.62	.....	47.51
B (NaOH)	1.976	92.72	26.470	62.30	.....	.....	13.40	.....
BA (HCl)	0.019	0.89	.....	.....	.....	.....	.....	.....
BAB (NaOH)	0.033	1.55	1.700	4.00	.....	.....	51.50	.....
BAB + H_2O	0.010	0.47	0.987	2.32	.....	.....	99.00	.....
Soil Residue	0.049	2.30	*13.320	*31.35	.....	.....	368.00	.....
Total	2.087	97.93	.....	*100.00	.....	.....	.....	.....

* By difference.

† Probably largely inorganic plant constituents.

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TABLE XVII

A SUMMARY OF THE DATA ON ALFALFA HAY

Total N in Material	3.798%	C/N
Total Organic C in Material	44.260%	11.65

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	C/N	Per cent C in Humus
	Per cent of Material	Per cent of Total N	Per cent of Material	Per cent of Total C				
A (HCl)	1.064	28.02	.....	.....	.....	.....	.....	.....
AB (NaOH)	2.500	65.83	16.51	37.31	.....	.....	6.61	.....
AB (NH_4OH)	.....	.....	14.73	33.29	29.40	0.84	.....	50.10
B (NH_4OH)	.....	.....	24.87	56.19	51.73	†6.06	.....	48.07
B (NaOH)	3.588	94.50	31.10	70.28	.....	.....	8.67	.....
BA (HCl)	0.052	1.37	.....	.....	.....	.....	.....	.....
BAB (NaOH)	0.053	1.40	2.40	5.42	.....	.....	45.30	.....
BAB + H_2O	0.008	0.21	2.01	4.54	.....	.....	251.00	.....
Soil Residue	0.028	0.74	*8.75	*19.77	.....	.....	312.00	.....
Total	3.729	98.22	.....	*100.00	.....	.....	.....	.....

* By difference.

† Probably largely inorganic plant constituents.

TABLE XVIII

A SUMMARY OF THE DATA ON SWEET FERN LEAVES AND ON OAK LEAVES

	Sweet Fern Leaves	Oak Leaves
Total N in Material	1.585	0.998
Total Organic C in Material	48.340	48.630
C/N	30.500	48.700

ANALYSIS OF THE SOLUTIONS

Treatment	Nitrogen		Carbon		Per cent Humus	Per cent Humus Ash	Per cent C in Humus
	Per cent of Material	Per cent of Total N	Per cent of Material	Per cent of Total C			
Sweet Fern Leaves:							
A (HCl)	0.103	6.50	.....	.....	.....	.....	.....
AB (NH_4OH)	.....	.....	15.64	32.35	31.81	3.20	49.17
B (NH_4OH)	.....	.....	21.54	44.55	41.70	2.46	51.65
Oak Leaves:							
A (HCl)	0.186	18.64	.....	.....	.....	.....	.....
AB (NH_4OH)	.....	.....	14.83	30.50	29.81	2.34	49.76
B (NH_4OH)	.....	.....	17.51	35.99	35.94	2.23	48.71

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TABLE XIX

A SUMMARY OF THE CARBON AND NITROGEN DATA IN TABLES III TO XVIII, SHOWING THE AVERAGE PERCENTAGES OF THE TOTAL NITROGEN AND TOTAL CARBON EXTRACTED BY THE DIFFERENT TREATMENTS IN SOILS, PEATS AND VEGETABLE MATERIALS

	A (HCl)	B (NH_4OH)	AB (NH_4OH)	AB (NaOH)	B (NaOH)	BAB (NaOH)	BAB + H_2O	Residue
Average per cent of Total N Extracted..								
8 Soils	3.17	.....	.....	68.53	64.69	14.11	8.34	14.91
4 Peats	3.78	.....	.....	72.91	72.99	10.44	5.28	11.03
3[1] and 5[1] Vegetable Materials	20.10	.....	.....	68.45	92.21	2.04	0.23	1.73
Average per cent of Total C Extracted..								
8 Soils	.....	24.91	51.27	58.52	45.30	18.97	15.26	20.47
4 Peats	.....	27.00	34.42	55.03	51.01	12.29	7.34	29.34
3[1] and 5[1] Vegetable Materials	.....	43.20	33.40	47.46	65.57	4.82	2.61	26.99

[1] Sweet Fern Leaves and Oak Leaves were not extracted by any of the NaOH treatments, but the results from these materials appear in A (HCl), AB (NH_4OH) and B (NH_4OH).

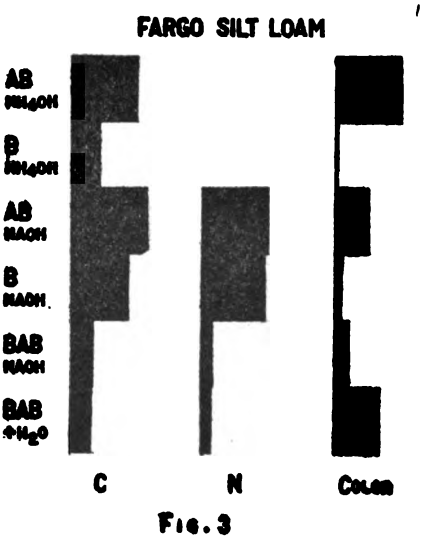

Fig. 2.—A graphic representation of the data on Fargo clay loam as given in Tables III and XXI.

Fig. 3.—A graphic representation of the data on Fargo silt loam as given in Tables IV and XXI.

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Fig. 4

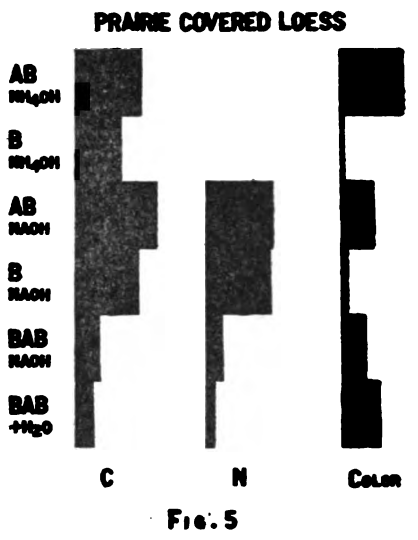

Fig. 5

Fig. 4.—A graphic representation of the data on Carrington silt loam as given in Tables V and XXI.

Fig. 5.—A graphic representation of the data on prairie-covered loess as given in Tables VI and XXI.

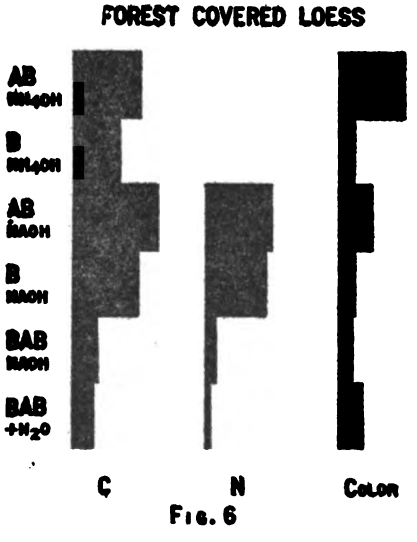

Fig. 6

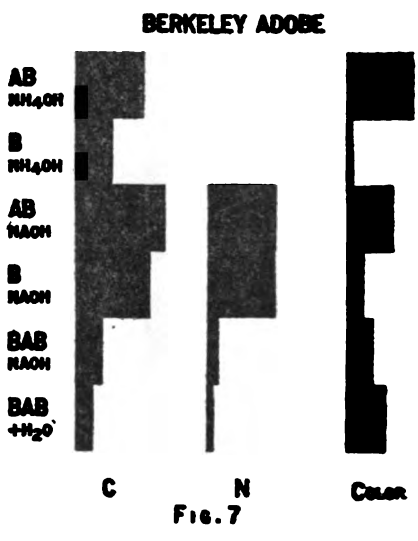

Fig. 7

Fig. 6.—A graphic representation of the data on forest-covered loess as given in Tables VII and XXI.

Fig. 7.—A graphic representation of the data on Berkeley adobe as given in Tables VIII and XXI.

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Discussion

I. *Is the humus extract of soils a typical soil product, formed in the soil by the action of bacteria and fungi, or can a similar extract be obtained from unchanged vegetable material?* From a study of the data which have been presented in the preceding tables it would seem that only one conclusion is possible, *i. e.*, the ordinary humus solution, AB (NH_4OH), contains to a very great extent dissolved substances which do not differ greatly in either color, carbon content, or nitrogen content

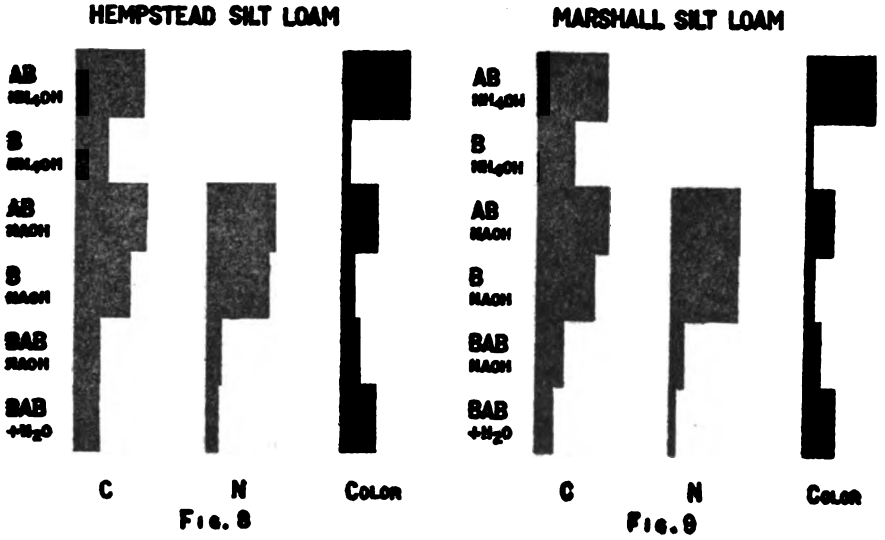

Fig. 8.—A graphic representation of the data on Hempstead silt loam as given in Tables IX and XXI.

Fig. 9.—A graphic representation of the data on Marshall silt loam as given in Tables X and XXI.

from similar substances which can be extracted by the same solvent from unchanged vegetable material. Table XXVI shows that a very considerable part of the ordinary humus is composed in reality of relatively colorless compounds. This fact is shown excellently by a reference to figures 2 to 10 in which carbon and nitrogen are compared with color. The carbon of B (NH_4OH) is undoubtedly largely present in AB (NH_4OH), only a small amount of carbon being soluble in the 1 per cent HCl.[1]

[1] In unpublished work the C/N ratio of the HCl extract was found to be approximately 14. The average percentage of nitrogen extracted from the eight soils by 1 per cent HCl was 0.00975 per cent of the soil, so that the average percentage of carbon extracted would be 0.140 per cent, or only 3.55 per cent of the (average) total carbon.

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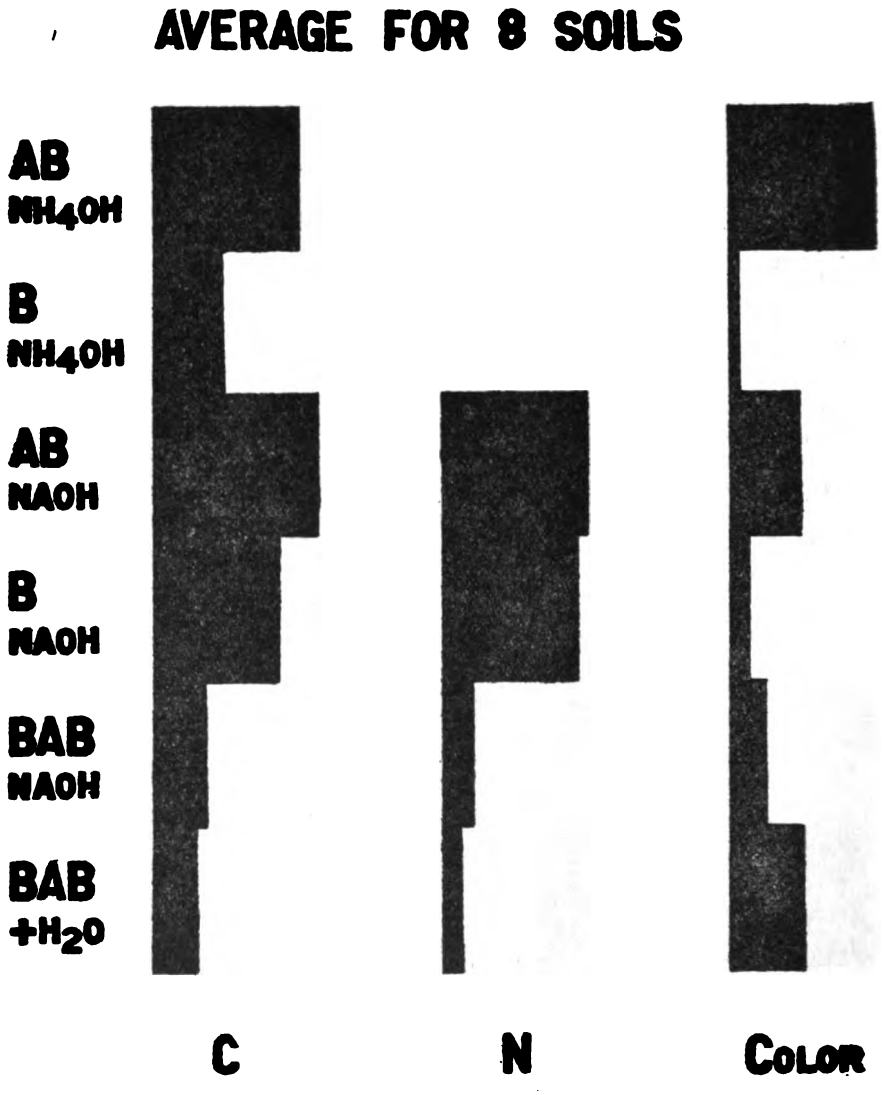

Fig. 10.—A graphic representation of the average data for the eight soils as given in Tables XIX and XXI.

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Fig. 11.—A graphic representation of the data on the acid muck soil as given in Tables XI and XXI.

Fig. 12.—A graphic representation of the data on sphagnum-covered peat as given in Tables XII and XXI.

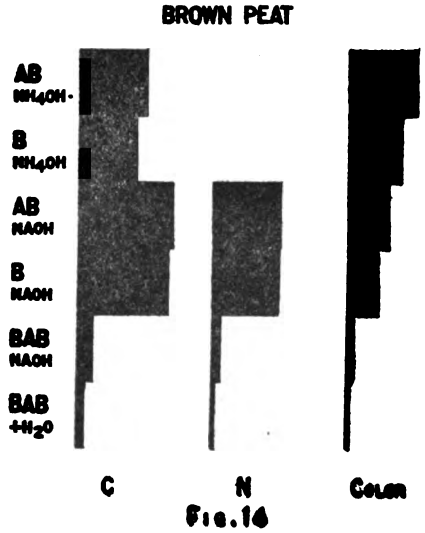

Fig. 13.—A graphic representation of the data on calcareous black peat as given in Tables XIII and XXI.

Fig. 14.—A graphic representation of the data on acid brown peat as given in Tables XIV and XXI.

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Whereas, however, the carbon shows an average increase of about 113 per cent from B(NH_4OH) to AB(NH_4OH) the color increases nearly 1400 per cent, and because of this the presence of a large proportion of colorless organic compounds has not been generally recognized.

The ordinary humus solution AB (NH_4OH) does contain the soil pigment, but as this is the only substance which appears with certainty to be a true soil product, and since the soil pigment probably rarely exceeds 35 to 40 per cent of the "humus," a determination of "humus" as ordinarily carried out, appears to be wholly without scientific justification.

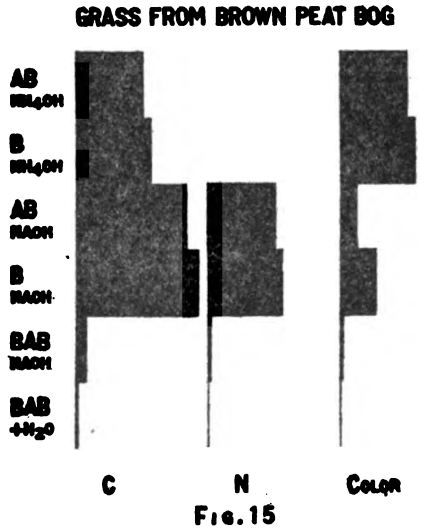

Fig. 15.

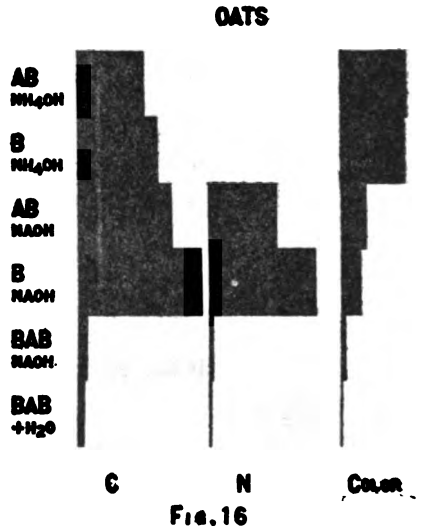

Fig. 16.

Fig. 15.—A graphic representation of the data on grass from brown peat bog as given in Tables XV and XXI.

Fig. 16.—A graphic representation of the data on the ripe oat plant as given in Tables XVI and XXI.

By no means all of the color of a "humus" solution is referable to the soil pigment, BAB + H_2O. The soil pigment gives a slaty black solution when viewed in a colorimeter and this black color cannot be used to match accurately the color of the NaOH solutions which do not dissolve the black pigment but which do dissolve a red-brown color. This deep red-brown color is not peculiar to soil organic matter but can be obtained in equal concentration from *dry, dead, oak leaves, which have never touched the ground.*

Another notable example is that afforded by the sphagnum-covered peat. "Humic acids" have been prepared from sphagnum peats for years and yet the range of color, carbon and nitrogen in the sphagnum-covered peat extracts differs in no essential detail from that in the

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TABLE XX

THE APPROXIMATE COLORS OF THE SOLUTIONS OBTAINED BY THE VARIOUS TREATMENTS

Soil or Vegetable Material Extracted	Treatment					
	B (NH_4OH)	AB (NH_4OH)	AB (NaOH)	B (NaOH)	BAB (NaOH)	BAB + H_2O
Fargo Silt Loam	very light terra-cotta	black	seal brown	terra-cotta	brown	black
Fargo Clay Loam	faint straw	seal brown	dark ochre	corn	light terra-cotta	coffee
Forest-covered Loess	light ochre	dark brown	ochre	light ochre	ecru	brownish gray
Prairie-covered Loess	light corn	seal brown	brown	dark corn	dark ochre	coffee
"Berkeley Adobe"	corn	seal brown	dark ochre	very light terra-cotta	ochre	light coffee
Hempstead Silt Loam	light ochre	brownish black	dark coffee	terra-cotta	very dark terra-cotta	seal brown
Carrington Silt Loam	ochre	brownish black	light seal brown	terra-cotta	light coffee	seal brown
Marshall Silt Loam	corn	brownish black	light brown	light ochre	gray-ochre	coffee
Sphagnum-covered Peat	seal brown	seal brown	dark terra-cotta	dark red-brown	light ochre	ecru
Black Peat	ochre	black	seal brown	seal brown	brown	slate black
Brown Peat	very dark brown	seal brown	dark brown	brown	light ochre	ecru
"Muck"	light ochre	light coffee	brown	ochre	very light ochre	brown-gray
Alfalfa Hay	orange yellow	very dark straw	straw	light yellow	colorless	colorless
Oats	ochre	ochre	orange-yellow	yellow-orange	faint straw	colorless
Grass from Brown Peat Bog	dark coffee	dark brown	light terra-cotta	terra-cotta	straw	colorless
Sweet Fern Leaves	dark seal brown	very dark brown	.....	.....	.....	.....
Oak Leaves	very dark brown	very dark brown	.....	.....	.....	.....

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unchanged vegetable materials. It seems to be impossible to reach any other conclusion but that this sphagnum-covered peat is, in all essential chemical relationships, a practically unchanged vegetable product, the only notable change which has taken place being a leaching out, or the decomposing, of a part of the easily soluble nitrogenous constituents [A (HCl) extract].

TABLE XXI

THE RELATIVE INTENSITY OF COLORATION OF THE SOLUTIONS WITHIN EACH SERIES AS MEASURED IN A COLORIMETER, AB (NH_4OH) BEING TAKEN AS UNITY

Soil or Vegetable Material Extracted	Treatment					
	AB(NH_4OH)	B(NH_4OH)	AB(NaOH)	B(NaOH)	BAB(NaOH)	BAB+H_2O
Fargo Silt Loam	1.00	20.00	2.00	9.60	4.16	1.47
Fargo Clay Loam	1.00	23.80	2.04	11.90	5.26	2.08
Forest-covered Loess	1.00	4.23	2.00	4.16	4.63	2.84
Prairie-covered Loess	1.00	20.00	2.00	10.70	3.03	1.70
Berkeley Adobe	1.00	9.43	1.51	4.31	2.73	1.81
Hempstead Silt Loam	1.00	9.43	1.92	5.35	3.70	1.96
Carrington Silt Loam	1.00	10.60	2.38	7.31	5.55	2.13
Marshall Silt Loam	1.00	13.80	2.50	8.06	4.41	2.17
Black Peat	1.00	10.80	2.08	2.50	4.54	2.77
"Muck"	1.00	3.24	1.35	2.72	3.78	2.12
Sphagnum-covered Peat	1.00	0.90	1.53	1.38	7.80	25.00
Brown Peat	1.00	1.25	1.56	2.13	10.00	20.00
Grass from Brown Peat Bog	1.00	0.91	4.31	1.96	26.30	colorless
Oats	1.00	1.03	2.63	3.33	17.00	colorless
Alfalfa Hay	1.00	0.82	1.43	2.50	colorless	colorless
Average for Soils (1-8)	1.00	13.91	2.04	7.67	4.18	2.02
Average for Peats (9-12)	1.00	4.05	1.63	2.18	6.53	12.49
Average for Vegetable Materials (13-15)	1.00	0.92	2.79	2.60	....	....

(The figures represent the number of cubic centimeters of solution required to produce the same intensity of color as that possessed by 1 c.c. AB (NH_4OH))

The "humus" from the sphagnum-covered peat, from the brown peat, as well as that from the unchanged vegetable materials shows no true black, but only the red-brown color which when in concentrated solution is so dark as to appear black. Alway and Pinckney (4) have observed that colorimetric methods for humus are reliable only when the standard humus solution used is prepared from a soil of the same locality, or soil type, as are the humus solutions which are to be measured. This is now easily explained because we have at least *three* variables which influence the color of the humus solution, AB(NH_4OH), containing a unit weight of humus; (1) a black pigment, peculiar to the soil, (2)

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a red-brown color extracted from dead plant materials, probably in an unchanged form and (3) a very considerable amount of practically colorless compounds. In addition to these we have still another phenomen, *i. e.*,

TABLE XXII

THE INTENSITY OF COLOR IN THE ORDINARY HUMUS SOLUTIONS, AB (NH_4OH), AS COMPARED BOTH COLORIMETRICALLY AND PHOTOMETRICALLY WITH A 0.0063 PER CENT SOLUTION OF THE (ASH FREE) "SOIL PIGMENT"; ALSO COMPARISONS OF THE AMOUNT OF SOIL PIGMENT WITH THE TOTAL ORGANIC MATERIAL PRESENT IN THE SOLUTIONS

Soil Solution Taken	Color Equals mg. "Soil Pigment" per c.c.			Mg. "Humus" per c.c. Actually Found (gravimetric)	Indicated Approximate Percentage of Soil Pigment in the "Humus Solution"	Actual Per cent of Carbon in the Humus Belonging to the Soil Pigment $\frac{AB\ (NH_4OH)\ Carbon}{BAB + H_2O\ Carbon}$
	Colorimetric	Photometric	Average			
AB (NH_4OH)						
Fargo Silt Loam	0.60	0.46	0.53	1.69	31	29.5
Fargo Clay Loam	0.22	0.14	0.18	0.51	35	30.0
Forest-covered Loess	0.11	0.08	0.10	0.35	28	28.5
Prairie-covered Loess	0.19	0.16	0.18	0.63	28	28.8
Berkeley Adobe	0.13	0.09	0.11	0.46	24	24.2
Hempstead Silt Loam	0.26	0.23	0.25	0.71	35	38.8
Carrington Silt Loam	0.35	0.28	0.32	0.93	34	29.2
Marshall Silt Loam	0.22	0.16	0.19	0.55	34	27.5
Black Peat	0.41	0.36	0.39	1.79	22	29.2
"Muck"	0.11	0.08	0.10	0.62	16	38.0
Sphagnum-covered Peat	*	0.15	....	2.06	None	None
Brown Peat	*	0.13	....	2.32	None	None
Grass from Brown Peat Bog	*	0.06	....	1.95	None	None
Oats	*	0.029†	....	1.66	None	None
Alfalfa	*	0.008†	....	1.84	None	None
Sweet Fern Leaves	*	0.07	....	2.09	None	None
Oak Leaves	*	0.12	....	1.91	None	None
B (NH_4OH)						
Sweet Fern Leaves	*	0.16	....	2.74	None	None
Oak Leaves	*	0.08	....	2.35	None	None

* It was impossible to read these solutions in a colorimeter against the soil pigment solution, inasmuch as they contained no black color, being merely more or less intense red brown.

† Not dark enough for photometric readings, values interpolated by comparing them colorimetrically with a sphagnum-covered peat AB (NH_4OH) standard.

colors produced by plant indicators. A large part of the color in the ammonia extract from the grass from the brown peat bog appears to be due to the presence of the hydroxyl ion. The hydrochloric acid extract of this grass was of a light straw color but the addition of ammonia caused

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TABLE XXIII

RELATIVE AMOUNTS OF ORGANIC CARBON IN EACH SOLUTION IN EACH SERIES, AB (NH_4OH) BEING TAKEN AS UNITY

Soil or Vegetable Material Taken	Treatment					
	AB(NH_4OH)	B(NH_4OH)	AB(NaOH)	B(NaOH)	BAB(NaOH)	BAB+H_2O
Fargo Silt Loam	1.00	2.25	0.86	1.13	3.14	3.16
Fargo Clay Loam	1.00	3.04	0.94	1.49	2.69	3.33
Forest-covered Loess	1.00	1.43	0.80	1.02	2.66	3.48
Prairie-covered Loess	1.00	2.46	0.84	1.12	2.16	3.48
Berkeley Adobe	1.00	1.92	0.76	0.91	2.51	4.10
Carrington Silt Loam	1.00	1.77	0.88	1.09	3.91	3.46
Hempstead Silt Loam	1.00	2.04	0.95	1.21	2.54	2.57
Marshall Silt Loam	1.00	1.89	0.96	1.17	2.51	3.65
Black Peat	1.00	2.26	0.54	0.65	1.99	3.40
"Muck"	1.00	1.31	0.58	0.69	2.06	2.61
Sphagnum-covered Peat	1.00	0.95	0.63	0.61	3.82	8.31
Brown Peat	1.00	1.16	0.71	0.74	4.13	8.04
Grass from Brown Peat Bog	1.00	0.90	0.61	0.55	7.00	36.00
Oats	1.00	0.83	0.71	0.54	8.47	14.50
Alfalfa	1.00	0.48	0.89	0.47	6.14	7.30
Average for Soils (1-8)	1.00	2.10	0.89	1.14	2.77	3.40
Average for Peats (9-12)	1.00	1.42	0.61	0.67	3.00	5.59
Average for Vegetable Materials (13-15)	1.00	0.74	0.74	0.52	7.20	19.26

(The figures represent the number of cubic centimeters of solution necessary to contain the same weight of organic carbon that is contained in 1 c.c. AB (NH_4OH))

TABLE XXIV

HUMUS AND HUMUS CARBON (FOUND AND CALCULATED) ON UNLEACHED SOILS

	Humus Per cent	Humus Carbon			
		Per cent Calc.[1]	Per cent Found	Error	Per cent Error
Marshall Silt Loam	1.49	0.745	0.791	—0.046	—6.1
Fargo Clay Loam	0.95	0.475	0.476	—0.001	—0.2
Fargo Silt Loam	3.65	1.825	1.775	+0.050	+2.8
Carrington Silt Loam	2.86	1.430	1.436	—0.006	—0.4
Prairie-covered Loess	1.42	0.710	0.702	+0.008	+1.1
Forest-covered Loess	1.16	0.580	0.570	+0.010	+1.7
Hempstead Silt Loam	2.00	1.000	0.929	+0.071	+7.6
Berkeley Adobe	1.24	0.621	0.658	—0.037	—5.6
Sphagnum-covered Peat	34.40	17.200	17.780	—0.580	—3.2
Black Peat	12.97	6.490	6.510	—0.020	—3.0
Brown Peat	35.29	17.640	17.360	+0.280	+1.6
"Muck"	5.82	2.910	2.814	+0.096	+3.4
Alfalfa	51.73	25.860	24.870	+0.990	+3.9
Oats	36.09	18.040	17.150	+0.890	+5.1
Grass from Brown Peat Bog	36.46	18.230	18.300	—0.070	—3.8
Sweet Fern Leaves	41.70	20.850	21.540	—0.690	—3.2
Oak Leaves	35.94	17.970	17.510	+0.460	+2.6

[1] Calculated by halving the humus percentages (humus carbon = $\frac{\text{humus}}{2}$).

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this solution to turn a quite dark red-brown which again became relatively colorless, without the formation of a precipitate, on the addition of acid. The hydrochloric acid extract of a soil cannot be tested in a similar manner because of the presence of iron and aluminium compounds which adsorb the indicator, but undoubtedly vegetable indicators are responsible for a part of the color in both ammonia and sodium hydroxide solutions.

All of the color data seem to indicate that, given the same materials ammonium hydroxide will dissolve *less* carbon and at the same time produce a *much darker solution* than will sodium hydroxide. Just why this

TABLE XXV

HUMUS AND HUMUS CARBON (FOUND AND CALCULATED) ON LEACHED SOILS

	Humus Per cent	Humus Carbon			
		Per cent Calc.[1]	Per cent Found	Error	Per cent Error
Marshall Silt Loam	2.850	1.425	1.510	—0.085	—5.6
Fargo Clay Loam	2.660	1.330	1.423	—0.093	—6.5
Fargo Silt Loam	9.910	4.955	5.174	—0.219	—4.2
Carrington Silt Loam	4.950	2.475	2.525	—0.050	—1.9
Prairie-covered Loess	3.400	1.700	1.737	—0.037	—2.1
Forest-covered Loess	1.795	0.897	0.821	+0.076	+9.2
Hempstead Silt Loam	3.610	1.805	1.865	—0.060	—3.2
Berkeley Adobe	2.390	1.195	1.260	—0.065	—5.1
Sphagnum-covered Peat	32.910	16.450	16.960	—0.510	—3.0
Black Peat	28.710	14.350	14.700	—0.350	—2.4
Brown Peat	39.220	19.610	20.140	—0.530	—2.6
"Muck"	7.140	3.570	3.715	—0.145	—3.9
Alfalfa	29.400	14.700	14.730	—0.030	—0.2
Oats	26.650	13.330	14.400	—0.930	—6.4
Grass from Brown Peat Bog	31.950	15.980	16.530	—0.550	—3.3
Sweet Fern Leaves	31.810	15.900	15.640	+0.260	+1.6
Oak Leaves	29.810	14.900	14.830	+0.070	+0.4

[1] Calculated by halving the humus percentages (humus carbon = $\frac{\text{humus}}{2}$).

should be the case it is difficult to state, but the color data for the vegetable materials in Table XXI show that the color of the ammonia AB (NH_4OH) solutions was more than twice as intense as that of the corresponding NaOH solutions. Hilgard (17, p. 125-126, 132) refers to NaOH as the more efficient "humifier" presumably meaning that it produces color from organic compounds more readily than does an ammonia solution so that the answer apparently does not lie in a chemical change. It is possible that a 4 per cent concentration of the fixed alkali is great enough to shift a color equilibrium. This could be tested relatively easily by measuring the intensity of coloration at different dilutions but has not been attempted in this connection.

II. *Does a 4 per cent sodium hydroxide solution extract the same substances from a soil as does a 4 per cent ammonium hydroxide solution, and if the same substances are extracted is the same quantity extracted by*

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each solvent? It is surprising that this question has not been answered long ago. Hilgard and Jaffa (18) originated a method for the determination of "humus nitrogen" in which the ammonia extraction of the "humus" was replaced by a sodium, or potassium hydroxide extraction, and nitrogen determined by Kjeldahling the resulting solution. Unless the same substances are extracted in the same amounts there is no justification for assuming that the nitrogen obtained by this process is a quantita-

TABLE XXVI

COMPARISON OF THE PERCENTAGES OF HUMUS OBTAINED FROM THE LEACHED SOIL AB (NH_4OH) WITH THOSE OBTAINED FROM THE UNLEACHED SOIL, B (NH_4OH) AND THE DIFFERENCE BETWEEN THESE VALUES WITH THE PERCENTAGES OF SOIL PIGMENT (BAB + H_2O CARBON × 2)

	Humus B(NH_4OH) Per cent	Humus AB(NH_4OH) Per cent	Increase	Approximate Per cent of "Soil Pigment" in Soil (BAB + H_2O C. × 2)	Difference Due to Relatively Colorless Compounds
Fargo Silt Loam	3.65	9.91	6.26	3.06	3.20
Fargo Clay Loam	0.95	2.66	1.71	0.86	0.85
Forest-covered Loess	1.16	1.80	0.64	0.47	0.17
Prairie-covered Loess	1.42	3.40	1.98	1.00	0.98
Berkeley Adobe	1.24	2.39	1.15	0.61	0.54
Hempstead Silt Loam	2.00	3.61	1.61	1.45	0.16
Carrington Silt Loam	2.86	4.95	2.19	1.47	0.72
Marshall Silt Loam	1.49	2.85	1.36	0.85	0.51
Average for Soils (1-8)	1.85	3.95	2.10	1.22	0.88
Black Peat	12.97	28.71	15.74	8.60	7.14
"Muck"	5.82	7.14	1.32	[1]2.83	....
Sphagnum-covered Peat	34.40	32.91	—1.49	None	....
Brown Peat	35.29	39.22	3.93	None	....
Grass from Brown Peat Bog	36.46	31.95	—4.51	None	....
Oats	36.09	26.65	—9.44	None	....
Alfalfa Hay	51.73	29.40	—22.33	None	....
Sweet Fern Leaves	41.70	31.81	—9.89	None	....
Oak Leaves	35.94	29.81	—6.13	None	....

[1] It is highly improbable that this is all "soil pigment," for the solution measured colorimetrically gave only 0.75 per cent soil pigment.

tive (or even qualitative) measure of the nitrogen in the "humus" obtained in the other process. Tables XX, XXI, XXIII, XXVII and XXVIII as well as figures 2 to 10 and the four figures on Plates I and II, show that no direct relationship exists. To be sure the carbon contents of AB (NH_4OH) and AB (NaOH) do not differ widely, the range being + 7.2 to + 38.5 per cent of humus, *in each instance in favor of the fixed alkali,* but the color of the extracts averages more than 100 per cent greater *in favor of the ammonia solution.* As for the nitrogen content of the two solutions, it is impossible to make a definite statement.

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Table XXX shows a comparison between a few nitrogen determinations run on the ammonia solutions and the corresponding sodium hydroxide extractions. Nitrogen was determined in the ammonia solution by evaporating 200 to 300 c.c. of the 4 per cent ammonia extract *in vacuo* at 45° to 50° C. after having first added about 10 c.c. of a 10 per cent $Ca(OH)_2$ suspension and continuing the evaporation *until the ammonia distilled off during 5 minutes was not sufficient to neutralize 4 drops of* $N/14$ H_2SO_4. The apparatus used for the distillation is the same as is used by Van Slyke (31) for the determination of ammonia in the hydrolysate of

TABLE XXVII

DIFFERENCE BETWEEN THE AMOUNTS OF ORGANIC MATTER EXTRACTED FROM THE UNLEACHED SOILS BY 4 PER CENT NaOH AND BY 4 PER CENT NH_4OH

Soil or Vegetable Material Extracted	Per cent Carbon B(NaOH)	Per cent "Humus" B(NaOH) (Calc.)[1]	Per cent "Humus" found B(NH_4OH)	Increase in favor of NaOH	Per cent Increase
Fargo Silt Loam	4.555	9.11	3.65	5.46	149
Fargo Clay Loam	0.979	1.96	0.95	1.01	106
Forest-covered Loess	0.796	1.59	1.16	0.43	37
Prairie-covered Loess	1.548	3.10	1.42	1.68	118
Berkeley Adobe	1.385	2.77	1.24	1.53	123
Hempstead Silt Loam	1.530	3.06	2.00	1.06	53
Carrington Silt Loam	2.349	4.70	2.86	1.84	64
Marshall Silt Loam	1.270	2.54	1.49	1.05	70
Black Peat	22.680	45.36	12.97	32.39	249
"Muck"	5.370	10.75	5.82	4.93	84
Sphagnum-covered Peat	27.400	54.80	34.40	20.40	59
Brown Peat	27.190	54.38	35.29	19.09	54
Grass from Brown Peat Bog	30.150	60.30	36.46	23.84	65
Oats	26.470	52.94	36.09	16.85	46
Alfalfa Hay	31.100	62.20	51.73	10.47	22
Average for Soils (1-8)	1.801	3.60	1.85	1.75	94
Average for Peats (9-12)	20.660	41.32	22.12	19.20	86
Average for Vegetable Materials (13-15)	29.240	58.48	41.43	17.05	41

[1] Calculated by multiplying the organic carbon by 2 (humus = humus carbon × 2).

proteins. The residue remaining in the flask after the removal of the ammonia was Kjeldahled as usual. The data are to meager for any generalization but they seem to indicate that a method has been found which can be utilized to determine the soil nitrogen in ammonia extracts with a fair degree of accuracy. No relationship between NaOH nitrogen and NH_4OH nitrogen is indicated by the data available.

In this connection it is of interest to compare the results which were obtained on Berkeley adobe with those obtained by Alway and Bishop (1). As already indicated, the present sample of soil was prepared by mixing their samples "A" and "B." Unfortunately the samples were not weighed before mixing so that an accurate comparison is impossible. Using the

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Rather method they obtained 1.71 per cent of humus for "A" and 1.19 per cent for "B." For the mixed soils is obtained by my methods 2.39 per cent, or considerably more than they obtained for their sample "A." They obtained 0.160 per cent and 0.119 per cent of "humus nitrogen" (by NaOH) while the writer obtained 0.159 per cent. Their "nitrogen in humus," obtained by using the AB (NaOH) figures for N and the AB (NH_4OH) figures for humus, were 9.3 per cent and 10.0 per cent. My value would be only 6.65 per cent.

TABLE XXVIII

DIFFERENCE BETWEEN THE AMOUNTS OF ORGANIC MATTER EXTRACTED FROM THE LEACHED SOILS BY 4 PER CENT NaOH AND BY 4 PER CENT NH_4OH

Soil or Vegetable Material Extracted	Per cent Carbon AB(NaOH)	Per cent "Humus" AB(NaOH) (Calc.)[1]	Per cent "Humus" Found AB(NH_4OH)	Increase in Favor of NaOH	Per cent Increase
Fargo Silt Loam	5.990	11.980	9.910	2.070	20.9
Fargo Clay Loam	1.537	3.070	2.660	0.410	15.4
Forest-covered Loess	1.018	2.040	1.800	0.240	13.3
Prairie-covered Loess	2.049	4.100	3.400	0.700	20.6
Berkeley Adobe	1.654	3.310	2.390	0.920	38.5
Hempstead Silt Loam	1.936	3.870	3.610	0.260	7.2
Carrington Silt Loam	2.890	5.780	4.950	0.830	16.7
Marshall Silt Loam	1.560	3.120	2.850	0.270	9.4
Black Peat	27.040	54.080	28.710	25.370	88.3
"Muck"	6.308	12.620	7.140	5.480	76.7
Sphagnum-covered Peat	26.200	52.400	32.910	19.490	59.2
Brown Peat	28.080	56.160	39.220	16.940	43.2
Grass from Brown Peat Bog	26.900	53.800	31.950	21.850	68.3
Oats	20.330	40.660	26.650	14.010	52.6
Alfalfa Hay	16.510	33.020	29.400	3.620	12.3
Average for Soils (1-8)	2.329	4.658	3.946	0.712	17.9
Average for Peats (9-12)	21.910	43.820	26.990	16.820	62.3
Average for Vegetable Materials (13-15)	21.250	42.490	29.330	13.160	44.8

[1] Calculated by multiplying the organic carbon by 2 (humus = humus carbon × 2).

These authors question Hilgard's generalization that the humus of arid soils is richer in nitrogen than is the humus from humid regions. This finding is substantiated by a comparison of the C/N ratios for AB (NaOH). There is no false comparison possible here, as in the case of comparing ammonia humus with NaOH nitrogen figures, and a low C/N ratio should indicate a high percentage of nitrogen in the humus. Tables XXXII shows the C/N ratios for both the leached and unleached soils. The typical humid, bottom land soil, Fargo silt loam, shows the highest content of nitrogen in the humus, AB (NaOH). Three of the soils contain even more nitrogen in proportion to the total carbon in the AB (NaOH) solutions than does the Berkeley adobe, while in five of the eight soils the B (NaOH) humus is richer in nitrogen than is the adobe.

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Of course, these data from a single sample are not conclusive proof that arid soils do not contain a humus which is richer in nitrogen than that obtained from soils of humid regions, but taken in connection with Alway and Bishop's (1) data it would seem to corroborate their conclusion that such a generalization is open to serious criticism.

TABLE XXIX

RELATIVE PERCENTAGES OF CARBON AND NITROGEN EXTRACTED FROM THE UNLEACHED SOIL BY 2 PER CENT NaOH AND BY 4 PER CENT NaOH, TOGETHER WITH C/N RATIOS

	Per cent Nitrogen			Per cent Carbon				
	2% NaOH	4% NaOH	Increase	2% NaOH	4% NaOH	Increase	C/N 2% NaOH	C/N 4% NaOH
Fargo Silt Loam	0.497	0.584	0.087	3.928	4.555	0.627	7.90	7.80
Fargo Clay Loam	0.102	0.127	0.025	0.787	0.979	0.192	7.71	7.71
Forest-covered Loess..	0.075	0.084	0.009	0.820	0.796	(–)0.024	10.93	9.50
Prairie-covered Loess.	0.166	0.186	0.020	1.374	1.548	0.174	8.18	8.32
Berkeley Adobe	0.141	0.157	0.016	1.268	1.385	0.117	8.99	8.82
Hempstead Silt Loam..	0.168	0.168	None	1.556	1.530	(–)0.026	9.26	9.10
Carrington Silt Loam.	0.246	0.271	0.025	2.274	2.349	0.075	9.24	8.67
Marshall Silt Loam...	0.145	0.152	0.007	1.114	1.270	0.156	7.68	8.36
Black Peat	1.863	2.146	0.283	18.980	22.680	3.700	10.18	10.57
Sphagnum-cove'd Peat	1.547	1.697	0.150	25.900	27.400	1.500	16.74	16.14
"Muck"	0.730	0.778	0.048	5.628	5.374	(–)0.254	7.71	6.91

TABLE XXX

PERCENTAGES OF SOIL NITROGEN EXTRACTED BY AMMONIUM HYDROXIDE COMPARED WITH THE AMOUNTS EXTRACTED BY SODIUM HYDROXIDE

	B (NH_4OH)			B (NaOH)	AB (NH_4OH)			AB (NaOH)
	I	II	Av.	Av.	I	II	Av.	Av.
Fargo Silt Loam	0.250	0.258	0.254	0.584	0.562	0.584	0.573	0.613
Marshall Silt Loam ..	.....	.....	.....	.....	0.194	.....	0.194	0.153
Hempstead Silt Loam.	0.145	0.142	0.144	0.168	.....	.....	.....	.....
Carrington Silt Loam.	0.215	0.249	0.232	0.271	.....	.....	.....	.....
Prairie-covered Loess..	0.117	0.127	0.122	0.186	.....	.....	.....	.....
Black Peat	0.889	0.869	0.879	2.146	.....	.....	.....	.....

As regards the solubility of the coloring material, Alway and Bishop (1) recently made the following statement:

"We have confirmed Hilgard and Jaffa's observation that after prolonged extraction of a soil with either ammonia or alkaline hydroxide solution the other fails to extract any appreciable amount of black material."

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Using the present methods the author is able to confirm their observation that, after prolonged extraction with ammonia, the 4 to 6 per cent sodium hydroxide solution fails to extract any appreciable amount of black material, but not the reverse. In every case the black pigment of

TABLE XXXI

RESULTS INDICATING THAT PERHAPS THE ORGANIC CARBON OF PEATS AND VEGETABLE MATERIALS CAN BE DETERMINED WITH A FAIRLY HIGH DEGREE OF ACCURACY BY DIVIDING THE VOLATILE MATTER BY AN EMPIRIC FACTOR

	Per cent "Volatile"	Per cent Carbon Calculated	Per cent Carbon Actually Found	Error	Per cent Error
		Volatile / 1.842			
Peats					
Black Peat	78.68	42.71	42.81	(—)0.10	(—)0.23
Brown Peat	86.10	46.74	46.34	+0.40	+0.86
Sphagnum-covered Peat	90.59	49.17	49.32	—0.15	—0.30
"Muck"	26.75	14.52	14.58	—0.06	—0.04
		Volatile / 1.971			
Vegetable Materials					
Alfalfa	88.47	44.88	44.26	+0.62	+1.40
Oats	86.29	43.78	42.51	+1.27	+2.98
Oak Leaves	94.13	47.75	48.63	—0.88	—1.80
Sweet Fern Leaves	92.44	46.90	48.34	—1.44	—2.97
Grass from Brown Peat Bog	92.97	47.17	47.02	+0.15	+0.32

TABLE XXXII

C/N RATIOS FOR THE LEACHED AND UNLEACHED SOIL EXTRACTS

	Fargo Clay Loam	Fargo Silt Loam	Carrington Silt Loam	Hempstead Silt Loam	Prairie-covered Loess	Forest-covered Loess	Marshall Silt Loam	Berkeley Adobe
AB (NaOH)	10.05	9.77	10.86	10.41	10.56	11.06	10.20	10.40
B (NaOH)	7.71	7.80	8.67	9.10	8.32	9.50	8.36	8.82
BAB (NaOH)	7.59	18.30	21.77	18.72	16.29	22.15	19.83	19.42
BAB + H_2O	15.29	19.13	23.03	23.35	20.00	31.20	31.92	23.46

the soil was found to be *quite insoluble* in 4 per cent NaOH, so much so that the addition of NaOH up to a 4 per cent concentration to a solution of the soil pigment in dilute alkali (0.1 per cent NaOH) causes a precipitation of the pigment.

The method of testing the solubility of the residue in ammonia after extraction with sodium hydroxide used by Alway and Bishop, as de-

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scribed to me by the former of these authors, and doubtless also that used by Hilgard and Jaffa, was the Hilgard-Jaffa method of extracting on a cone-shaped filter, with a protecting disk of filter paper, adding the extracting fluid in small portions until the filtrate became practically colorless (*cf.* Alway, Files, and Pinckney, [3] 1910).

Taking the Marshall silt loam, which was from the same field as the humid soil that Alway and Bishop used, it was leached on a filter, following a previous leaching with HCl, with 4 per cent NaOH until the solution came through colorless and then leached, without removing it from the filter, with 4 per cent NH_4OH. The first 100 c.c. were almost colorless and even the first day's leachings, totaling about 500 c.c., were not black as had been expected, but light brown. The second and third day's leachings, totaling about 300 c.c. were, however, intensely black, and further leachings rapidly became colorless. *Had the author not been certain that a soluble pigment remained in the soil residue after the NaOH treatment, he should probably have not leached with more than* 50 *to* 100 *c c. of ammonia, and thus have arrived at the same conclusion as previous investigators.* It is strange that the pigment does not dissolve in the first portions of the ammonia poured through the filter. It may be that the defloculated soil is at first impervious to the ammonia solution and that the filtrate obtained is simply what passes between the soil mass and the filter without penetrating the soil. The sodium hydroxide in the external part of the soil mass probably has for some time a sufficient concentration to prevent a solution of the soil pigment which dissolves in the presence of NaOH only when the concentration of the fixed alkali is very low.

When instead of extracting with ammonia on a funnel, the residue from the NaOH extraction was placed in a beaker, or cylinder, ammonia added and the soil mass thoroughly disintegrated, after which the clay was floculated with ammonium carbonate, the solution was found to be intensely black.

These observations serve to explain the erroneous conclusions of Hilgard and Jaffa, of Alway and Bishop, and possibly of numerous other investigators who have not published their confirmation of Hilgard and Jaffa's observations.

The following experiments were made in order to test out quantitatively the author's observations as to a difference in solubility in the two solvents. Two portions of 15 gm. each, of the same soil type (Fargo silt loam), but differing in that one contained only 0.20 per cent of carbonate CO_2 while the other contained 9.90 per cent were leached with 1 per cent HCl, and extracted in the shaking machine three successive periods of 4 days each with fresh quantities of 750 c.c. 4 per cent NH_4OH. "Humus" was determined on each extract. Similar extractions were made, with the use of successive portions of 4 per cent NaOH. At the end of the three

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extractions the contents of the bottles were acidified with HCl, the precipitated soil washed first by decantation and later on a filter until free from chlorides, and then 4 per cent NaOH was added to the residue from the NH_4OH treatment, and NH_4OH to the residue from the NaOH extractions. The final portion of sodium hydroxide did not dissolve any black color but the solution became a quite intense red-brown while the final ammonium hydroxide solution was *jet black*. Table XXXIII and figure 17 show the amount of

TABLE XXXIII

AMOUNTS OF CARBON, "HUMUS" AND COLOR OBTAINED FROM TWO SOILS IN THREE SUCCESSIVE EXTRACTIONS WITH 4 PER CENT NH_4OH, FOLLOWED BY A SINGLE EXTRACTION WITH 4 PER CENT NaOH, AS WELL AS THE REVERSE SERIES OF THREE EXTRACTIONS WITH 4 PER CENT NaOH FOLLOWED BY A SINGLE EXTRACTION WITH 4 PER CENT NH_4OH

	Soil 1. Organic C. 10.02% Carbonate CO_2 0.20%				Soil 2. Organic C. 10.03% Carbonate CO_2 9.90%			
	Per cent Carbon	Per cent Humus	Per cent Humus Ash	Color[1]	Per cent Carbon	Per cent Humus	Per cent Humus Ash	Color[1]
1st extr. NaOH.....	5.58	....	....	350	4.99	....	....	340
2nd extr. NaOH.....	0.71	....	....	33	0.80	....	....	42
3rd extr. NaOH.....	0.26	....	....	14	0.32	....	....	15
Residue + NH_4OH...	[2]1.16	2.33	0.63	467	[2]1.40	2.80	0.37	540
Ist extr. NH_4OH....	2.60	5.21	0.41	966	3.16	6.33	[3]1.16	1066
2nd extr. NH_4OH....	0.69	1.37	0.42	62	0.72	1.44	1.09	76
3rd extr. NH_4OH....	0.37	0.74	0.23	32	0.39	0.78	0.86	30
Residue + NaOH....	1.61	....	....	63	1.88	....	....	63

[1] Color is referred to a 0.0063 per cent solution of the ash-free soil pigment, a ratio of 100 equalling the color of this pigment solution, while a ratio of 1000 would equal the color of a 0.063 per cent solution.

[2] The carbon figures for NH_4OH solutions are obtained by dividing the humus by 2 (cf. Tables XXIV and XXV).

[3] The high "humus ash" figures in this column do not seem to be due to suspended clay but to dissolved mineral matter. This is the only soil I have found which always gives a high humus ash. The solubility continues practically the same through three successive extractions with NH_4OH.

carbon, "humus" and color actually obtained on the different solutions. It can be readily seen that these data agree in all respects with the conclusions indicated by the preceding tables and discussions, *i. e., Sodium hydroxide does not dissolve either the same substances or the same quantity of substances from a soil as does ammonium hydroxide.*

III. *Is the soil nitrogen present in a different form from that in which the nitrogen occurs in plant materials, or does the nitrogen of the soil exhibit the same solubilities as vegetable nitrogen?*

From a study of the preceding tables it becomes evident that the nitrogen of the soil is not exactly similar to that of unchanged vegetable ma-

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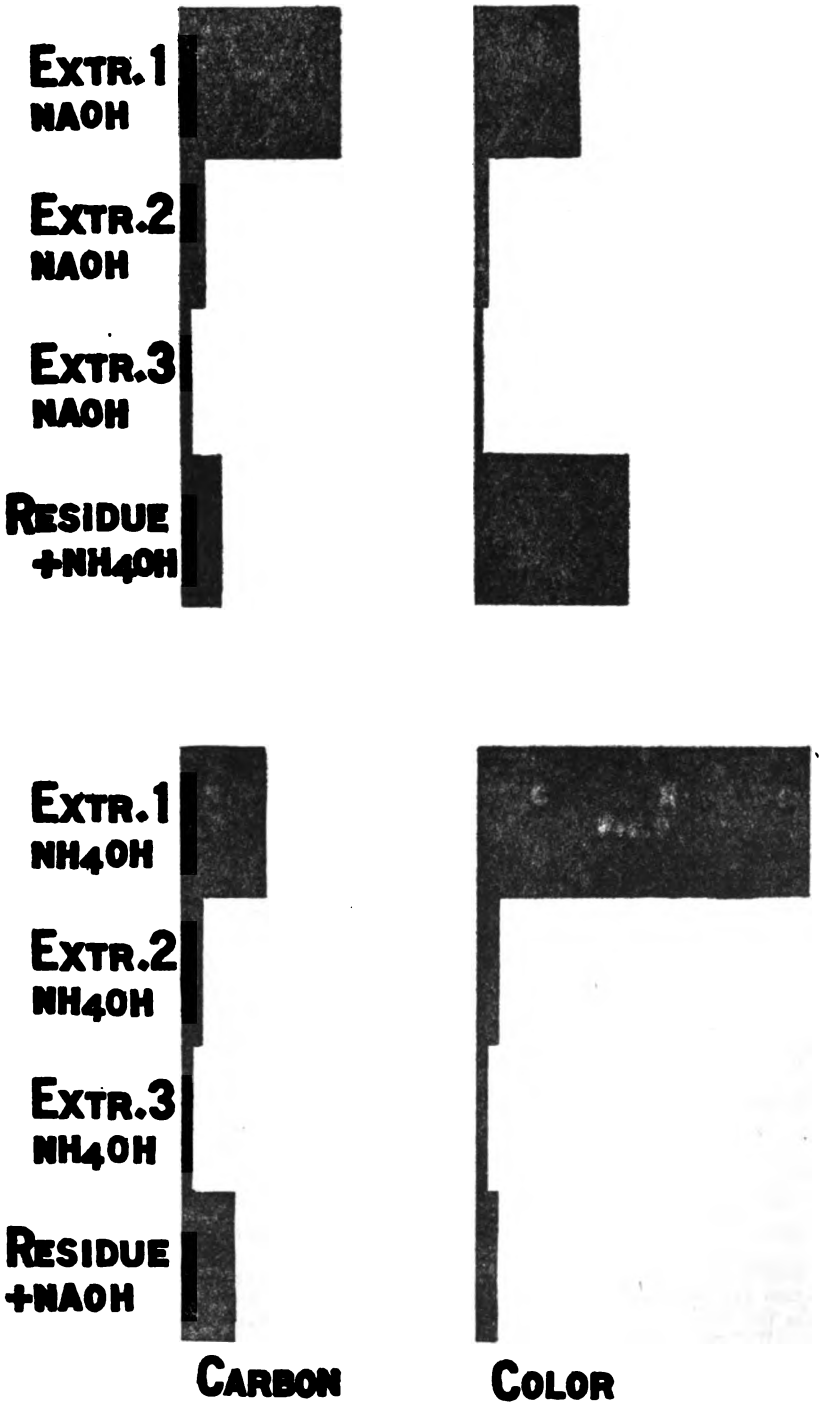

Fig. 17.—A graphic representation of the average of the determinations given in Table XXXIII.

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terials. Summary Table XIX shows the comparison. The principal difference seems to be that the easily soluble forms of nitrogen, such as dissolve in 1 per cent HCl, do not long remain in the soil, while, on the other hand, there is a considerable amount of nitrogen in the soil which exhibits marked insolubility in NaOH solutions, in decided contrast to the behavior of the vegetable materials tested. It seems possible that the insoluble nitrogen may represent, at least in part, the nitrogen of the spores of bacteria and fungi. The easily soluble nitrogen is apparently very readily broken down in the soil to ammonia and nitrates, or possibly it may leach out.

It is of interest to note that no very large portion of the soil nitrogen is rendered insoluble by the lime and magnesia of the soil. Potter and Snyder (24) call attention to the necessity of leaching soils in order to set free the proteins from their calcium salts and thus render the proteins soluble. A reference to Table XIX shows that, for the average of the eight soils, leaching increased the amount of nitrogen dissolved less than 4 per cent, the color increasing, however (Table XXI), 376 per cent. In other words, the substances which impart color to the soil contain only a small percentage of nitrogen, the average ratio of C/N in the soil pigment (BAB+H_2O) being 22.6 for the eight soils or, assuming that the materials contain 50 per cent of carbon, a nitrogen content of only 2.21 per cent. It thus appears to be a coincidence that the darker soils from the same locality are, in general, the more fertile.

It will be noted that two of the acid peats contain none of the soil pigment while the "acid muck" contains but very little. These soils present very unfavorable media for bacterial growth; indeed, it has long been recognized that acid peat bogs are practically free from bacteria at no great distance from the surface. On the other hand the calcareous peat contains a large amount of the black pigment.

Beijerinck (7) found that *Azotobacter chroococcum* excretes a dark brown or black pigment and Hall (15, p. 188) suggests that this pigment may play its part in the coloration of humus. It may well be that BAB + H_2O, the soil pigment, is produced by a beneficial bacterium. Its presence and amount would accordingly be an indication of the bacterial activity and thus the quantity present would be roughly proportional to the soil fertility. Unfortunately, it was impossible to secure bacteriological data on the soils used, but it is to be hoped that some investigator will attempt to answer the problem: *Is the black pigment a bacterial product?*[1]

[1] Since the manuscript of this paper was completed a paper has appeared by Briggs, Jensen and McLane (8), in which they present data showing that there is a high degree of correlation between the "humus" content of the soil and "mottle-leaf" of citrus trees. Their humus percentages were determined colorimetrically against a standard humus solution.

Alway and Pinckney (4) and Alway and Blish (2) have shown that the colorimetric method for humus is approximately accurate only when the standard humus solution is prepared from a soil of the same soil type and locality as the sample under investigation. Kelly and McGeorge (19,

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IV. *The Relationship of carbon to humus.* After all of the humus carbon determinations had been made it was found that humus carbon can be determined within experimental error by halving the humus figures. This is shown in Tables XXIV and XXV. It seems probable that the carbon content of the "unleached humus" is slightly lower than 50 per cent while that of the "leached humus" is slightly above 50 per cent, but the slight differences are in practically all cases within experimental error. The fact that the same factor applies to the ammonia extracts from peats and unchanged vegetable materials, speaks very strongly in support of the contention that the soil organic matter does not differ to any marked extent from that of unchanged plant materials and that a specific "humification," giving rise to a *humus* in which the nitrogen is more readily available for plant-food than in unchanged vegetable materials does not take place.

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p. 18), dealing with Hawaiian soils, mention a series of "humus" solutions which were of a yellowish straw color" but which gave a gravimetric humus percentage exceeding 3 per cent. Alway and Pinckney (4, p. 4) state that they could not obtain satisfactory results when the colorimetric method was applied to a number of desert soils.

As the author has shown earlier in this paper, there are four variables which influence the color of the solution containing a unit weight of humus, *i. e.*, the amount of black soil pigment, the amount of red-brown plant coloring materials, the amount and nature of the vegetable indicators, and the proportion of colorless compounds present in the ammonia extract. The range of variation in these factors is beautifully illustrated by some data on page 243 of Alway and Blish's (2) paper. These authors were working with the very uniform soil type, the loess, the extremes differing mainly in rainfall. They find an average of 0.33 and 0.31 per cent of humus (gravimetric) respectively for the 3 to 6-foot section in the "Wauneta" and "Weeping Water" areas, while by colorimetric methods they obtain 0.52 per cent for the "Wauneta" area and 0.08 per cent for the "Weeping Water" area, *or a difference of* 500 *per cent by the colorimetric method on soils having an almost identical humus content.*

Briggs, Jensen and McLane (8) present evidence in their own paper which proves that certain of their humus determinations are unreliable. It is exceedingly improbable that the ratio of carbon to organic matter ever rises above 2. The European factor for the total organic matter of sols is carbon x 1.724 (20, p. 15), which is decidedly lower than the 1.842 which I found for peats and 1.971 for unchanged vegetable materials (*cf.* Table XXXI). Using the European factor, we find that in soils Nos. 100, 123, 124 and 127, Briggs *et al* obtain a greater percentage of humus than there was total organic matter in the soil. Hilgard (17, p. 132) states that this factor (1.724) will give results which are from 40 to 50 per cent higher than the humus content. or, in other words, that the ammonia-soluble materials represent from 50 to 60 per cent of the total organic matter. If this be the case, a very considerable proportion of the "humus" determinations of Briggs *et al* are much too high. These authors fail to find a correlation in the case of the lemon groves between humus and mottle-leaf. In this instance the soil is all from the same locality and presumably from the same soil type, so that the (relative) humus percentages should be quite accurate, provided they were all matched against the same standard. The criticism of the use of the colorimetric method would, therefore, not apply with equal force in these experiments.

Inasmuch as these authors have apparently not recognized the weaknesses of the colorimetric method, the accuracy of their humus determinations is open to serious question. Whether the errors are great enough to vitiate entirely their correlations can be determined only by actual experiment, in which the humus is determined gravimetrically.

It does appear possible, however, that there is a positive correlation between *soil pigment* and mottle-leaf. It seems to me to be extremely improbable that this soil pigment plays any part in the nutrition of the citrus trees or the prevention of mottle-leaf, *per se.* The low nitrogen content, and the persistence of the soil pigment at considerable depth in the soil, seem to indicate that the soil pigment is not readily destroyed. May it not be possible, if the positive correlation between the color of the ammonia extract of soils and mottle-leaf of citrus trees holds true, that mottle-leaf is due to decreased bacterial activity in the soil, the amount of soil pigment being merely an indicator of bacterial activity?

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Having shown that the humus contains approximately 50 per cent of carbon, a direct comparison of the "humus" extracted by NaOH and NH_4OH is possible. This is shown in Tables XXVII and XXVIII and in every instance sodium hydroxide extracted more humus than the ammonia.

It was thought possible after the carbon-humus ratio had been determined that a ratio might be found by which the "volatile matter" of peats and vegetable materials could be transposed into carbon. Such an attempt on a small scale is shown in Table XXXI. The factors used are the average factors for the determinations given. It will be noted again that the factor for the unchanged vegetable materials is practically 2, which would mean that the carbon content of the undissolved material is approximately the same as that of the "humus" dissolved by 4 per cent NH_4OH. It is to be hoped that someone will extend this series and see if the factors given are actually reliable over a large number of determinations.

V. · *A comparison of the carbon and nitrogen dissolved from unleached soils by 2 per cent and by 4 per cent sodium hydroxide solutions.* It was the original intention to investigate the amounts of carbon dissolved by different concentrations of sodium and ammonium hydroxides. Interest, however, became diverted to other directions early in the progress of the work, but not until the data were practically complete for two concentrations of sodium hydroxide on the unleached soils. These are shown in Table XXIX. The 2 per cent NaOH solution has been used almost exclusively in the study of the organic matter of the soil carried out by the Bureau of Soils of the U. S. Department of Agriculture. The data available indicate that 2 per cent NaOH dissolves the same substances (*cf.* C/N ratios) in almost the same amount as does a stronger solution. However, the length of time during which the solution was in contact with the soil and the thoroughness of the stirring are factors which operate greatly to equalize the amount dissolved.

Summary

Eight mineral soils, three peats, one muck and five samples of unchanged vegetable materials were selected and analyzed for total carbon and total nitrogen; for carbon and "humus" soluble in 4 per cent NH_4OH both before and after leaching the substances with 1 per cent HCl; for carbon and nitrogen soluble in 4 per cent NaOH both before and after leaching the material with 1 per cent HCl; for carbon and nitrogen extracted by 4 per cent NaOH from the residue remaining after the soil or vegetable materials which, without previous treatment, had been extracted with 4 per cent NaOH and subsequently leached with acid; for carbon and nitrogen extracted by the addition of water to the residue remaining from the preceding extraction and for nitrogen in the 1 per cent HCl ex-

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tract. Colorimetric measurements were also made on the various solutions.

From a comparison of the data obtained in this series of analyses, the following conclusions are evident:

1. The "humus" extract of soils and peats is not a typical soil product, formed in the soil by the action of bacteria and fungi, for very similar extracts can be obtained from unchanged vegetable materials.

2. "Humus," the "matière noire" of Grandeau, does not consist entirely of a black compound, or compounds, but it also contains a large proportion of almost colorless substances, the presence of which is normally masked by the black color.

3. A 4 per cent solution of sodium hydroxide does not extract either the same substances or the same quantity of substances as does a 4 per cent solution of ammonium hydroxide.

4. From a given soil, after leaching with 1 per cent HCl, ammonium hydroxide will extract *less carbon* and at the same time *more color* than will a sodium hydroxide solution.

5. In general the forms of soil nitrogen appear to be quite similar in solubility to those forms occurring in unchanged vegetable materials, the only noteworthy difference being that vegetable materials contain a very considerable amount of nitrogen soluble in 1 per cent HCl while soils contain only a small quantity, and, on the other hand, the soils contain an appreciable amount of nitrogen quite insoluble in 4 per cent NaOH, in decided contrast to the unchanged vegetable materials.

6. All the mineral soils and the one calcareous peat tested, contained a *soil pigment* which is absent from the unchanged vegetable materials or the acid peats.

7. This soil pigment appears to be the only substance which can, with certainty, be said to be a true soil product. It is suggested that perhaps the soil pigment is of bacterial origin.

8. This soil pigment is intensely black, and contains only a relatively small proportion of the soil nitrogen. Therefore, it would appear to have but little importance, *per se*, in the problem of soil fertility.

9. It is extremely doubtful whether a specific "humification" of plant materials takes place in the soil.

10. Inasmuch as sodium hydroxide does not dissolve either the same substances or the same quantity of substances as does ammonium hydroxide, the common practice of comparing "humus nitrogen" determined in a 4 per cent NaOH extract, with "humus," obtained by 4 per cent NH_4OH, can give only meaningless results.

11. Inasmuch as the "humus" extract of soils is undoubtedly a mixture of organic compounds, many of which are colorless and in all probability are extracted from unchanged plant or animal materials, and inasmuch as the soil pigment present in this solution probably rarely exceeds

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40 per cent of the "humus," a determination of the "humus" as ordinarily carried out, appears to be wholly without scientific justification. The European method of reporting "humus" by making a determination of total organic carbon, appears to be far preferable.

Literature Cited

(1) Alway, F. J., and Bishop, E. S.
1916. Nitrogen content of the humus of arid soils. *In* Jour. Agr. Research, v. 5, p. 909-916.

(2) Alway, F. J., and Blish, M. J.
1916. The loess soils of the Nebraska portion of the transition region. II, Humus, humus-nitrogen and color. *In* Soil Sci. v. 1, p. 239-258.

(3) Alway, F. J., Files, E. K., and Pinckney, R. M.
1910. The determination of humus. *In* Jour. Indus. Engin. Chem., v. 2, p. 317-322.

(4) Alway, F. J., and Pinckney, R. M.
1912. The photometric and the colorimetric determinations of humus. *In* Nebr. Agr. Exp. Sta. 25th Ann. Rpt. (1911), p. 2-16.

(5) Ames, J. W., and Gaither, E. W.
1914. Determination of carbon in soils and soil extracts. *In* Jour. Indus. Engin. Chem., v. 6, p. 561-564.

(6) Association of Official Agricultural Chemists.
1912. Official and provisional methods of the association of official agricultural chemists. *In* U. S. Dept. Agr. Bur. Chem. Bul. 107 (revised).

(7) Beijerinck, M. W.
1901. Ueber oligonitrophile Mikroben. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 7, p. 561-582.

(8) Briggs, L. J., Jensen, C. A., and McLane, J. W.
1916. Mottle-leaf of citrus trees in relation to soil conditions. *In* Jour. Agr. Research, v. 6, p. 721-739, 3 pl.

(9) Burgess, J. L., and Worthen, E. L.
1906. Soil survey of Lancaster County, Nebraska. *In* U. S. Dept. Agr. Bur. Soils Field Operations, 1906, p. 943-962, 1 fig., 1 map.

(10) Burke, R. T. A., and Kolbe, L. A.
1909. Soil survey of Rice County, Minnesota. *In* U. S. Dept. Agr. Bur. Soils Field Operations, 1909, p. 1269-1303, 1 fig., 1 map.

(11) Funk and Wagnalls Co.
1913. New Standard Dictionary of the English Language. 2916 p., 58 pl. New York.

(12) Gortner, R. A.
1916. A rapid method for the estimation of calcium oxide in peat soils. *In* Soil Sci. v. 1, p. 505-508.

(13) Grandeau, Louis.
1872. Recherches sur le rôle des matières organiques du sol dans les phénomèes de la nutrition des végétaux. *In* Compt. Rend. Acad. Sci. [Paris], t. 74, p. 988-991.

(14) Grandeau, Louis.
1877. Traite d' Analyse des Matières Agricoles. 487 p., 46 fig. Paris.

(15) Hall, A. D.
1913. The Soil. 2nd ed. 311 p. E. P. Dutton & Co. New York.

Digitized by Google

(16) HILGARD, E. W.
1894. The chemical and physical investigation of soils. *In* Jour. Amer. Chem. Soc., v. 16, p. 34.

(17) HILGARD, E. W.
1907. Soils, their Formation, Properties, Composition and Relations to Climate and Plant Growth in the Humid and Arid Regions. 593 p. The MacMillan Co. New York.

(18) HILGARD, E. W., and JAFFA, M. E.
1894. On the nitrogen contents of soil humus in the arid and humid regions. *In* Cal. Agr. Exp. Sta. Rpt. 1894-95, p. 35-36.

(19) KELLEY, W. P., and MCGEORGE, W.
1911-12. A study of humus in Hawaiian soils. Hawaii Agr. Exp. Sta. Press Bul. 33, 23 p.

(20) KÖNIG, J.
1911. Untersuchung landwirtschaftlich und gewerblich wichtiger Stoffe. Aufl. 4. 1226 p., 426 fig. Paul Parey, Berlin.

(21) LYON, T. L., and BIZZELL, J. A.
1910. Effect of steam sterilization on the water-soluble matter in soils. N. Y. (Cornell) Agr. Exp. Sta. Bul. 275.

(22) MANGUM, A. W., and SCHROEDER, F. C.
1906. Soil survey of the Crookston Area, Minnesota. *In* U. S. Dept. Agr. Bur. Soils Field Operations 1906, p. 865-891, 1 fig. 1 map.

(23) MOORES, C. A., and HAMPTON, H. H.
1908. The separation of clay in the estimation of humus. *In* Jour. Amer. Chem. Soc., v. 30, p. 805-807.

(24) POTTER, R. S., and SNYDER, R. S.
1916. Soluble non-protein nitrogen of soil. *In* Jour. Agr. Research, v. 6, p. 61-64.

(25) RATHER, J. B.
1911. An improved method for the estimation of humus. *In* Jour. Indus. Engin. Chem., v. 3, p. 660-662.

(26) SCHREINER, O., and LATHROP, E. C.
1912. The Chemistry of steam heated soils. U. S. Dept. Agr. Bur. Soils Bul. 89, 37 p.

(27) SEAVER, F. J., and CLARK, E. D.
1910. Studies on pyrophilous fungi. II. Changes brought about by the heating of soils and their relation to the growth of *Pyronema* and other fungi. *In* Mycologia, Bd. 2, p. 109-124.

(28) SEAVER, F. J., and CLARK, E. D.
1912. Biochemical studies of soils subjected to dry heat. *In* Biochem. Bul., v. 1, p. 413-427.

(29) SMITH, W. G., and KIRK, N. M.
1914. Soil survey of Ramsey County, Minnesota. *In* U. S. Dept. Agr. Bur. Soils Field Operations 1914, Separate, 37 p., 2 fig., 1 map.

(30) TRUOG, E.
1915. Methods for the determination of carbon dioxide and a new form of absorption tower adapted to the titrametric method. *In* Jour. Indus. Engin. Chem., v. 7, p. 1045-1049.

(31) VAN SLYKE, D. D.
1911. The analysis of proteins by determination of the chemical groups characteristic of the different amino acids. *In* Jour. Biol. Chem., v. 10, p. 15-55.

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PLATE I

Fig. 1.—Showing the relative intensity of coloration *per unit weight of organic carbon* in the different extracts from Fargo clay loam. Each tube contained 0.0042 gm. organic carbon per 50 c.c. volume.

Fig. 2.—Showing the relative intensity of coloration *per unit weight of organic carbon* in the different extracts from Fargo silt loam. Each tube contained 0.0046 gm. organic carbon per 50 c.c. volume.

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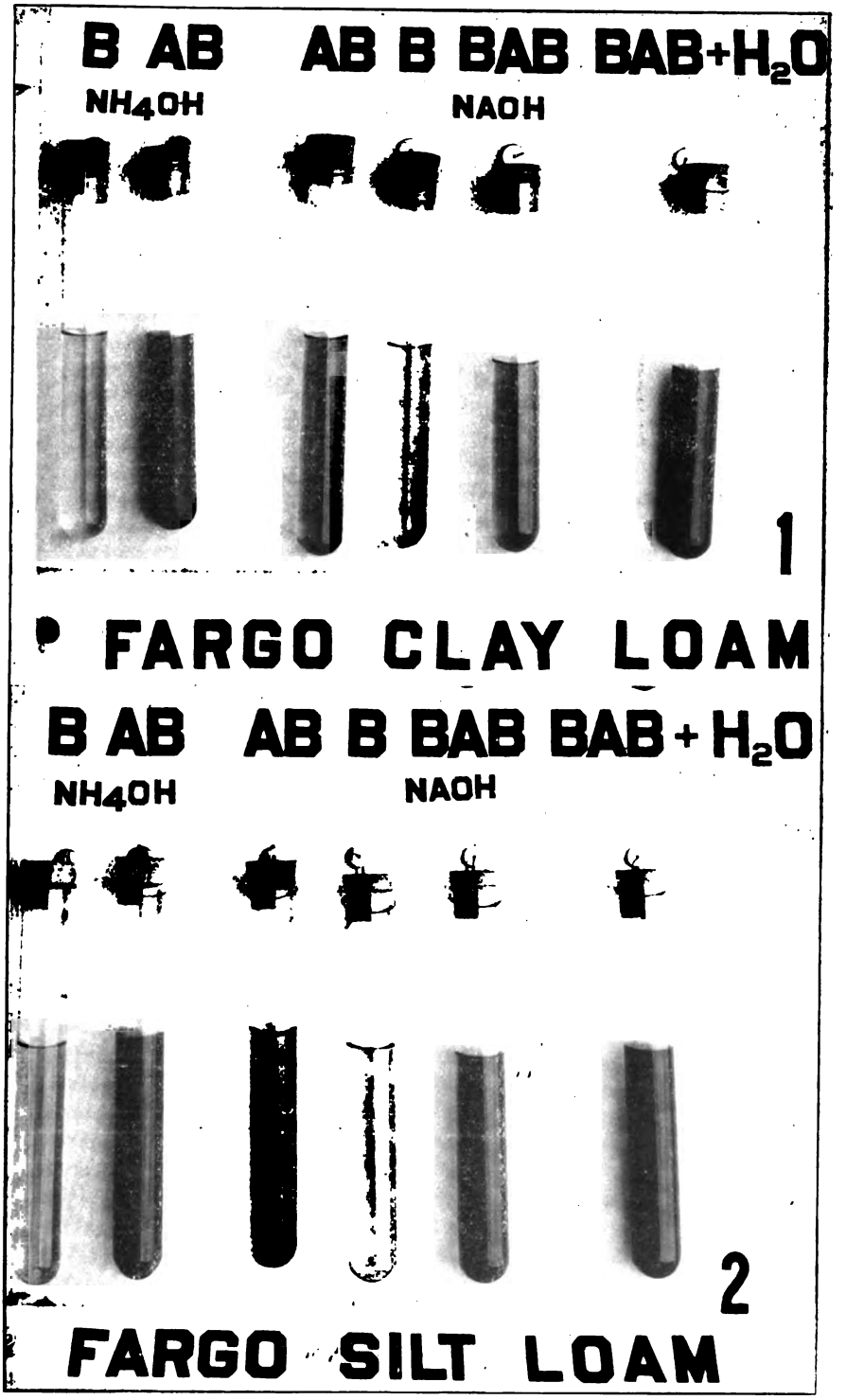

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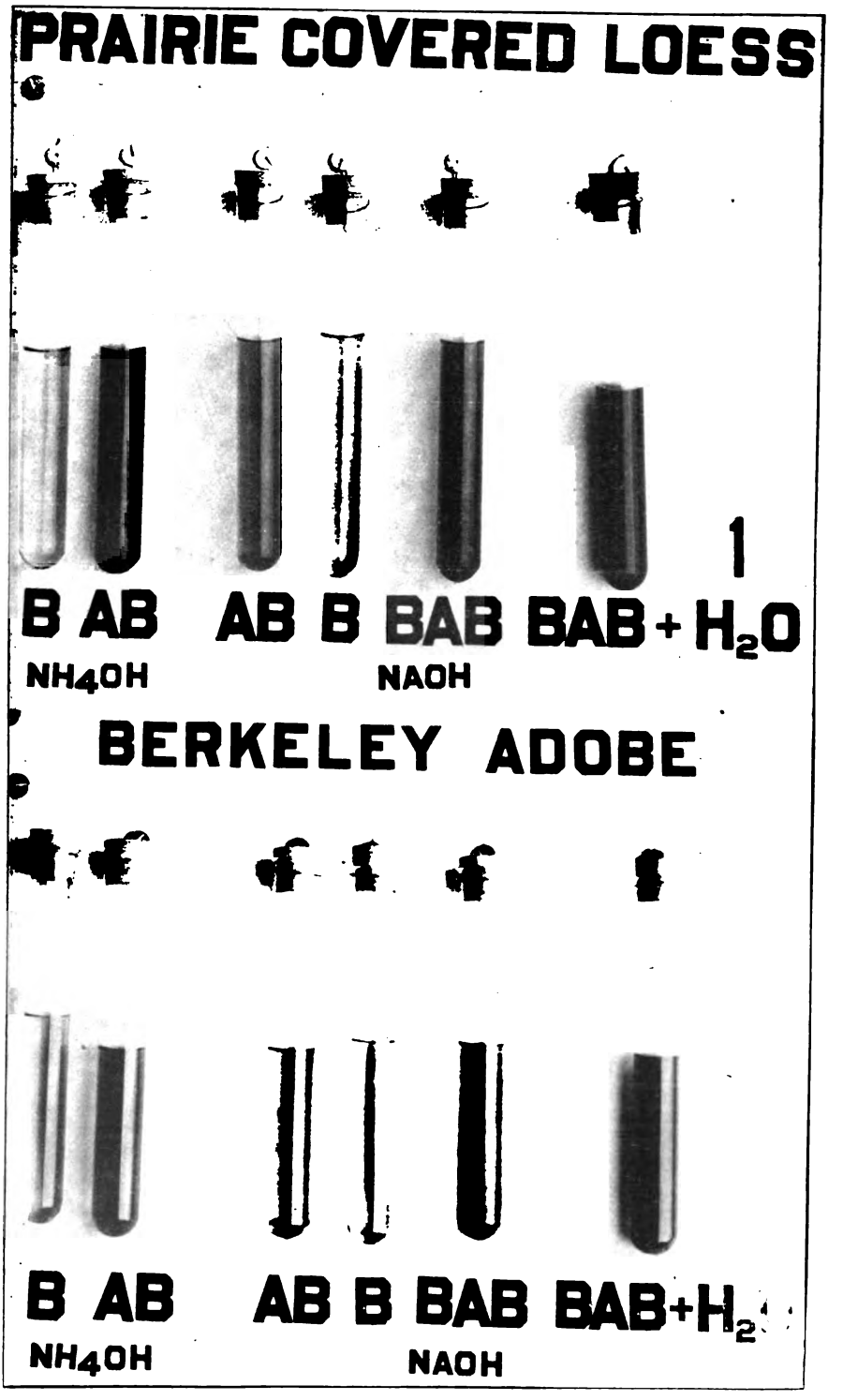

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PLATE II

Fig. 1.—Showing the relative intensity of coloration *per unit weight of organic carbon* in the different extracts from prairie-covered loess. Each tube contained 0.0046 gm. organic carbon per 50 c.c. volume.

Fig. 2.—Showing the relative intensity of coloration *per unit weight of organic carbon* in the different extracts from Berkeley adobe. Each tube contained 0.0030 gm. organic carbon per 50 c.c. volume.

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THE INFLUENCE OF SALTS ON THE BACTERIAL ACTIVITIES OF THE SOIL[1]

By

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Salts which may occur in soils and those applied to them in various operations influence the number, species, and activity of the microflora of a soil. These factors are in turn reflected by the yields obtained from the soil. Some substances applied to a soil serve as food for the growing plant; others increase plant growth but not through the direct furnishing of food. This latter effect may be due to a change brought about by the salt on the physical, chemical, or bacterial properties of the soil. The substance may alter the physical properties of the soil to such an extent that the bacterial flora is modified; this in turn may increase or decrease the crop produced upon the soil. Other substances may react chemically with constituents within the soil and in so doing liberate substances which can be directly utilized by the growing plant. Again, they may directly modify the microflora and fauna of the soil both as to numbers and physiological efficiency. Or, in some cases all three changes may be wrought by one and the same salt. The question, therefore, arises as to what effect this or that fertilizer or soil amendment is going to have upon the bacterial activity of the soil. Furthermore, there are millions of acres of land in arid America which contain varying amounts of soluble salts. Some of these soils contain such large quantities of these so-called "alkalies" that no vegetation is found upon them. Other soils contain only a medium amount of soluble salts and the vegetation is composed chiefly of alkali-resisting plants. Still other soils contain much smaller quantities of soluble salts and they become injurious only when the soil is improperly handled. The reclaiming of the heavily charged soils and the maintaining of the others in a productive condition can be carried on successfully only when we understand the influence of salts upon the growing plants and their action upon the biological, chemical, and physical properties of the soil.

[1] Contribution from the chemical and bacteriological departments of the Utah Agricultural Experiment Station.

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The purpose of this investigation is, therefore, to determine the relative toxicity of various substances found in or applied to a soil, as measured in terms of bacterial activities of the soil, also to determine the stimulating influence of various substances upon bacterial activity and the manner in which this stimulation is exerted. The results thus obtained can be directly compared with those obtained for higher plants. Then, if a correlation between the two (the lower and the higher plants) be obtained, it should give a quick method of testing not only alkali soils, but also other soils containing various soluble constituents. Furthermore, it makes possible the studying of balanced solutions within the soil by means of bacteriological tests, thus getting in a short time comparative results which with higher plants would be obtained only by an enormous amount of work and time. Knowledge thus obtained can be used in the reclaiming of the alkali lands of the arid West. A careful review of the literature has been made, and below is given the more recent important work dealing with the action of various salts upon the bacterial flora of the soil.

Historical

Much work has been done to determine the influence of calcium carbonate, especially when applied to acid soils, on the bacterial content and activity of the soil, but the conclusions reached have not always been concordant. Withers and Fraps (56) found that calcium carbonate added to a soil greatly accelerated nitrification and that it is especially desirable that it should be added where ammonium sulphate is being used as a fertilizer. Lipman's (32) work showed that calcium carbonate stimulated nitrification more than did gypsum, and that sodium chloride was injurious to nitrifying organisms, while ferrous sulphate in amounts from 10 to 100 mg. per 100 gm. of soil was without effect. Later, he and Brown (34) decided that both ammonification and nitrification were promoted by magnesia lime to a more marked extent than they were by non-magnesia lime. This, however was to a certain extent dependent upon the treatment and crop growing on the soil. Both ammonification and nitrification were accelerated by sodium nitrate. In a more recent work Lipman, Brown and Owen (36) found that small applications of calcium carbonate stimulated bacterial activity, but large applications had a detrimental effect upon ammonification.

In Owen's (44) experiments, magnesium carbonate was more efficient in promoting ammonification and nitrification than was either calcium or potassium carbonate. According to Engberding (10) ammonium sulphate, sodium nitrate, potassium nitrate and caustic lime all increase the bacterial content of the soil, but decrease its nitrogen-fixing powers. Krueger's (23) work indicated that calcium carbonate was more effective in promoting nitrification than was lime, the reverse was true with regard

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to the putrefactive bacteria. The formation of ammonia from peptone was especially favored by calcium carbonate. Lyon and Bizzel (38) found that lime favored nitrification, as did also certain nodule-bearing legumes. Fischer (12) concluded that the presence of calcium carbonate in a nutritive solution favored the formation of protein nitrogen, while magnesium carbonate lessened the transformation of ammonia into protein nitrogen. Calcium oxide, however, exerted a much greater influence upon soil bacteria than did calcium carbonate.

Kellerman and Robinson's (20) results are of especial interest as they indicate that magnesium carbonate, applied in amounts exceeding 0.25 per cent to a soil fairly high in magnesium carbonate, positively inhibited nitrification, while calcium carbonate up to 2 per cent favored it, thus indicating that the lime-magnesia ratio is of great importance with regard to bacteria as well as the higher plants. These results have been confirmed by C. B. Lipman and Burgess (30a) in whose experiments magnesium carbonate was very toxic both in soil and solution to *Azotobacter chroococcum,* while calcium carbonate was never toxic even in quantities up to 2 per cent. Furthermore, calcium carbonate exerted a protective influence against the toxic properties of magnesium carbonate. The optimum ratio varied, depending upon the media.

Peck (46) studied the influence of a number of salts upon bacterial activity when applied to the soil, with the result that the carbonate, sulphate and phosphate of calcium were found to stimulate ammonification, while sodium nitrate depressed it; both potassium sulphate and calcium carbonate accelerated nitrification in soil. Brown (3) working with a typical Wisconsin drift soil found that the application of ground lime up to 3 tons per acre increased the number of bacteria in the soil, also the ammonifying, nitrifying and nitrogen-fixing powers of the soil. The increase was in every case nearly proportional to the limestone applied.

At times the increase noted in ammonification is due to the retention of the volatile ammonia by the carbonate, as is shown by Lemmermann's (24) results where the addition of calcium carbonate to a soil up to 1 per cent reduced the volatilization of ammonia, but calcium oxide had the opposite effect. Both calcium chloride and calcium sulphate reduced the loss of ammonia, but the chloride was the only salt of magnesium tested which had this effect. Potassium and sodium, chloride, sulphates and carbonates all reduced the absorptive powers of the soil. Paterson (45) studied the influence of a number of substances upon nitrification with the result that caustic lime was found practically to stop all nitrification. Calcium carbonate promoted it, as did also magnesium carbonate; gypsum was less effective, while ferric hydrate had a very favorable effect. Sodium chloride, on the other hand, had a distinctly injurious effect.

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Kelly (21) studied the effect of calcium and magnesium carbonate alone and in combination upon ammonification and nitrification. In his work calcium carbonate only slightly stimulated ammonification of dried blood, but it had a marked stimulating effect upon nitrification. The magnesium carbonate was found to be toxic to both groups of organisms. No antagonism was found to exist between calcium and magnesium. Later, when working with Hawaiian soils, he (22) reports a stimulation for both. The results, however, varied with different soils and he considers the lime-magnesia ratio of little importance as regards the ammonifying and nitrifying organisms. Allen's (1) conclusion is that large quantities of limestone must be applied to a non-calcarious soil in order to bring its nitrifying powers up to those of natural calcareous soils.

Peterson (47) and Wollny (57) found that lime increased the carbon dioxide given off from soils, and Ebermeyer (8), Hilgard (19), and Hartwell and Kellogg (18) proved conclusively that lime increases the decay taking place in a soil.

Chester (4, 5) showed that lime increased the number of bacteria in soil, the increase being proportional to the lime applied up to 4,000 pounds per acre. He considered the effect as being due to the lime giving to the soil a more favorable reaction for the growth of bacteria and not to its direct action upon the organisms themselves.

These findings were later confirmed by Fabricicus and von Feilitzen (11), Engberding (10), Ehrenberg (9) and Fischer (12).

Lime not only increases the number of organisms in a soil, but it increases the ammonifying powers of the soil as is seen from the work of Remy (49), Ehrenberg (9) and Voorhees and Lipman (54).

The literature dealing with the influence of lime upon the nitrifying organisms is voluminous. No attempt is made here to refer to all of it, for in most cases the experiments were conducted on soils which were acid and the lime supplied neutralized the acidity of the soil, thus give the necessary neutral reaction for the action of the nitrifying organisms. Such results give little, if any, idea of the direct stimulating or toxic influence of calcium. Furthermore, the work has recently been summarized by Brown (3) who concludes that the application of lime increased nitrate production from ammonium sulphate and dried blood, the gain being almost proportional to the quantity of lime applied. This, in turn, was found to bear a close relationship to the number of organisms developing on synthetic agar.

Gypsum is a strong soil stimulant and in most cases it greatly increases the crop yield. This beneficial effect may be due to its liberation of potash or to the supplying of sulphur for the direct nutrition of the plant. At other times it may react with the ammonia formed by the ammonifying organisms, the neutral ammonium sulphate so formed being

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readily nitrified. There is also the possibility that calcium sulphate acts as a direct stimulant to the microörganisms of the soil. The literature is meager and inconclusive on this phase of the subject.

Severin (52) concludes from his work that gypsum not only prevents the loss of ammonia from manure, but it increases the speed of decomposition from 10 to 20 per cent, while Paterson (45) states that gypsum slightly increases nitrification in soil as determined by laboratory experiments. Prior to this, Pichard (48) had shown that the sulphates of calcium, potassium, and sodium promote nitrification.

Opposite results are reported by Dezani (6) who found that gypsum in amounts varying from 0.5 to 2 per cent had no appreciable effect on nitrification, while the results obtained by Lipman et al (33) varied and were inconclusive.

According to Lipman (25) calcium chloride in solutions accelerated the action of ammonifiers, and it is interesting to note that in a later work he (26) failed to find antagonism between either calcium and magnesium or calcium and sodium. The chlorides of calcium, magnesium, potassium, and sodium were found to be toxic in the order named. Marked antagonism exists between calcium and potassium, magnesium and sodium, and potassium and sodium. Sea water was found to be a physiologically balanced solution for *Bacillus subtilis*.

Many writers have made great claims for iron sulphate as a fertilizer. A goodly number of these have been made by individuals who would profit by its sale, but even when we ignore these cases there are still many cases in which it has produced good results. The composition of the crop usually indicates that iron sulphate influences the phosphorus (14) metabolism of the plant, and it is hard to see how this is possible unless it be that the iron stimulates the bacterial activity, which in turn liberates phosphorous from its insoluble forms within the soil. It has already been noted that Lipman (32) found ferrous sulphate to have but little effect upon nitrification, but his results were not conclusive. Guffroy (16) found sulphate of iron decidedly beneficial to oats, less beneficial in the case of rye, without action on rye grass and harmful to wheat. He concluded that its action must be due to its influence on the biological processes of the soil. According to Paterson and Scott (45) ferric hydrate has a distinctly beneficial effect upon nitrification. In this latter case its action could be due to its serving as a base. According to Lipman and Burgess (30) the ammonifiers are more sensitive to iron sulphate than are the nitrifiers, for while the latter were stimulated by small amounts of iron sulphate it was toxic to the former in all concentrations tested.

Magnesium compounds usually stimulate bacterial activities to a greater extent than do calcium compounds, as has been noted in some of the literature already cited, but Engberding's (10) results showed that,

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while magnesium sulphate stimulated bacterial activities, it was not as effective in this regard as was ammonium sulphate, sodium nitrate, or potassium sulphate. The work of Makrinov (39) is of interest as he found pure magnesium carbonate a very suitable substance on which to grow the nitrous organism. Furthermore, magnesium carbonate had a very beneficial effect on the physiological action of the organism. Kellerman and Robinson (20), on the other hand, found that magnesium carbonate when applied to a soil already rich in magnesium carbonate positively inhibited nitrification if the quantity added exceeded 0.25 per cent. We have here an apparent contradiction, but it may be due to the different conditions of the experiments, as one investigator was working with cultures of the organisms while the other was using the soil with its complex flora. Furthermore, it is quite possible that magnesium carbonate may be without effect upon or even accelerate the growth and activity of the nitrosomonas and yet inhibit the nitrobacter.

Manganese stimulates higher plants and in many cases acts as a positive catalyzer. Skinner and Sullivan (53) conclude from some of their experiments that manganese salts increase the oxidation in some soils. But while Montanan (42) found that manganese carbonate, sulphate, and dioxide greatly stimulated nitrification he attributed it to either the direct or the indirect furnishing of oxygen to the nitrifying organisms, and not to any catalytic effect of the manganese itself. Beijerinck (2) observes that some soil organisms have the power of oxidizing manganous carbonate. Olaru (43) found that manganese in the proportion of one part to 200,000 parts of nutritive media greatly increases nitrogen fixation and he considers it quite likely that the increased yield obtained after the application of manganese compounds to a soil is due to its accelerating the action of the nitrogen-fixing organisms of the soil.

Potassium is essential for the nutrition of both the higher and lower forms of plant life. Hence, it is to be expected that when added to a medium poor in potassium it will increase bacterial growth, but like many other true nutrients may become toxic if present in too great a concentration. As already noted, Engberding (10) states that potassium sulphate caused a slight increase in the bacterial content of a soil. While Peck (46) found it to increase nitrification in soils, Renault (50) claims that slow ammonification and subsequent nitrification is always accompanied by a low percentage of potash. Dumont's (7) experiments showed that potassium carbonate, added to a soil at the rate of from 1 to 2.5 gm. per 1,000 gm. of soil, markedly increased nitrification, but that larger applications of the salt progressively diminished the rate of nitrification, while the addition of 8 gm. to 1,000 gm. of soil completely checked it. Lumia (37) concluded that potassium chloride and sulphate were nearly as effective in promoting the activity of alcoholic ferments as were phosphates.

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Fred and Hart (13) found that both calcium and potassium sulphates increased ammonification in solution and that the sulphates of potassium, calcium and magnesium each increased the evolution of carbon dioxide from soil. But from the results obtained with different salts, they conclude that the addition of the potassium ion did not materially increase ammonification in the soil examined.

Sodium salts are often used as fertilizers and with good results. Furthermore, many alkali soils contain sodium salts in quantities sufficient to be toxic to both the higher and the lower plants. For these reasons many investigations have been conducted to determine the influence of sodium compounds upon higher plants, and a considerable number have had as their object the determination of their influence upon soil bacteria.

As early as 1884 Warington (55) showed that the presence of 0.032 per cent of sodium bicarbonate distinctly retarded nitrification, and that in the presence of 0.096 per cent nitrification was very slight. Schlösing (51) had added various salts to the soil in quantities not exceeding 485 parts per million with no apparent effect upon nitrification. However, Deherain found that common salt commenced to be harmful when it exceeded one-thousandth of the weight of soil, and when larger quantities are applied nitrification almost ceased. According to the same observer sodium nitrate may stop nitrification for a time, but later it recommences. Lipman (32) showed that there was a distinct diminution of nitrates produced as the application of sodium chloride was increased, and when 0.1 per cent was added nitrification was greatly diminished. Later he (35) and others found that sodium nitrate increased the accumulation of nitrates in a soil. They found, however, a certain periodicity in the accumulation of nitrates which would account for the different results reported by various investigators, and in later investigations they (33) concluded that at times sodium nitrate stimulates ammonification. McBeth and Wright (40) found that carbonates, chlorides and sulphates inhibited nitrification and that the former were more injurious than the latter.

The most far-reaching and systematic work which has been reported on the influence of salts upon bacterial activity is the excellent work by C. B. Lipman (27) who demonstrated that ammonification is inhibited by sodium chloride, sodium sulphate and sodium carbonate. The points at which the salts became toxic are: for sodium chloride, between 0.1 per cent and 0.2 per cent; for sodium sulphate, 0.4 per cent; and for sodium carbonate, 2.0 per cent. A stimulating influence was noted in the case of sodium carbonate, but not in the case of the sulphate or of the chloride. The points at which they became toxic to nitrifiers (28) were found to be: for sodium carbonate, 0.025 per cent; for sodium sulphate, 0.35 per cent; and for sodium chloride, less than 0.1 per cent.

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All except the carbonate acted as a stimulant in lower concentrations. Later Lipman and Sharp (31) found the point at which sodium chloride became toxic to nitrogen-fixing organisms in soil to be from 0.5 to 0.6 per cent; sodium sulphate, at 1.25 per cent; and sodium carbonate, at 0.4 to 0.5 per cent. Sodium chloride was the only one which acted as a stimulant. Recently Lipman (29) has demonstrated that there exists, as measured by ammonification, a true antagonism between sodium chloride and sodium sulphate; between sodium chloride and sodium carbonate; and between sodium sulphate and sodium carbonate.

At times the stimulating effect, or even the toxic influence, may be due to the liberation of substances within the molecule of the salt, as for example, in the case of the sulphates it may be the sulphur, but no attempt has been made in this summary to cover the literature on this important phase of the subject.

Experimental

The soil used in this work was that of the College Farm which is of a sedimentary nature. It was deposited by streams flowing into the valley laden with debris derived from the nearby mountains, which are composed largely of quartzite and limestone. A physical and chemical analysis of the soil is given in Table I.

TABLE I

PHYSICAL AND CHEMICAL COMPOSITION OF SOIL

Physical	Per cent	Chemical	Per cent
Coarse Sand (above 1 mm.)	17.69	Insoluble Matter	66.69
Fine Sand (1 to 0.03 mm.)	37.39	Potash (K_2O)	0.55
Coarse Silt (0.03 to 0.01 mm.)	15.19	Soda (Na_2O)	0.49
Medium Silt (0.01 to 0.003 mm.)	10.36	Lime (CaO)	7.41
Fine Silt (.003 to 0.001 mm.)	10.32	Magnesia (MgO)	4.15
Clay (below .001 mm.)		Ferric Oxide (Fe_2O_3)	2.93
Moisture and Loss	9.05	Alumina (Al_2O_3)	3.49
		Phosphoric Pentoxid (P_2O_5)	0.25
		Sulphur Trioxid (SO_3)	0.07
		Carbon Dioxid (CO_2)	7.62
		Humus	2.18
		Total Nitrogen	0.15

The soil used, therefore, was a sandy loam very high in acid-soluble constituents, but the water-soluble constituents were not excessive. The calcium and magnesium contents were very high and mainly in the form of the carbonate. The soil was well supplied with phosphorus and potassium and there was a fairly large quantity of iron present. In fact all of the elements of plant-food were present in abundance, with the exception of nitrogen which was low. The soil was very productive and previous work had shown the ammonifying and nitrifying powers of the soil to be about the average for the soils of the arid regions. The nitrogen-fixing powers of the soil were above the average and previous work had shown it to have an intensely interesting bacterial flora.

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Several hundred pounds of the soil were thoroughly mixed, stored in a large box, and kept as near field conditions as possible so that all the work could be done on the same soil. As the soil was needed in the work, portions were brought to the laboratory, air-dried in the dark, then weighed in 100-gm. portions into sterile covered tumblers. To each of these was added 2 gm. of dried blood. The whole was then carefully mixed and the salt in most cases added from a carefully standardized stock solution. This, together with sufficient sterile distilled water to make the moisture content up to 18 per cent, was thoroughly mixed in the soil. Each series, together with sterile blanks, was incubated at 28° to 30° C. for 4 days and then the ammonia determined by adding magnesium oxide and distilling into standard acid. In every case at least four determinations were made with each concentration of the salt, and, in the absence of agreement between determinations, the series was repeated so that the results as herein reported are in every case the average of four or more closely agreeing determinations. Hence, experimental error has been reduced to as near a minimum as possible in this kind of work.

The solutions of the salts were prepared by weighing gram-molecular quantities of Merck's best grade of the respective salts into 1,000 c.c. of sterile distilled water and then quantitatively determining the amount present. In those cases in which the analysis showed the concentration wrong, it was corrected, so that we have a definite knowledge of the quantity of salt added to the soil as the varying results reported by different investigators can in many cases be interpretated by the unknown variation in salts added. The solution thus prepared was then added to the soil in such quantities as to make the amount of the anion and of the cation the same and directly comparable the one with the other. The comparatively insoluble salts—calcium carbonate, calcium sulphate, etc.—were carefully weighed and intimately mixed with the soil. The arranging of the work in this order gives us as nearly absolute results as can be obtained by the present bacteriological methods, and at the same time gives us directly comparable results, which after all is what we have to look for in this work.

The salts tested were the chlorides, nitrates, sulphates, and carbonates of sodium, potassium, calcium, magnesium, manganese, and iron.

Ammonification

Influence of Sodium Salts: The compounds used in this series were sodium chloride, sodium sulphate, sodium nitrate and sodium carbonate. They were used in concentrations such that equivalent quantities of sodium in the various forms could be directly compared. The strengths varied from 0 to 5060.4 parts per million of soil, and represented the actual proportion of sodium in the various forms applied to the soil.

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The results are reported in the form of per cent, considering the ammonia produced in the untreated soil in each case as 100. This method of reporting the results makes them more directly comparable than if stated as milligrams of ammonia formed in 100 gm. of soil. It did not, however, materially change the numerical values, as the average ammonifying power of the untreated soil was 98 mg. of ammonia per 100 gm. of soil. The results are given in Table II and in every case are the average of at least four and sometimes of several times this number of closely agreeing determinations; so they should represent very closely the comparative influence of the various sodium salts upon ammonification.

TABLE II

PERCENTAGES OF AMMONIA FORMED IN 100 GRAMS OF SOIL CONTAINING 2 GRAMS OF DRIED BLOOD AND VARYING AMOUNTS AND FORMS OF SODIUM SALTS, THE UNTREATED SOIL TAKEN AS 100 PER CENT

Sodium—Parts per Million	Per cent of Ammonia Formed in the Presence of			
	NaCl	Na_2SO_4	$NaNO_3$	Na_2CO_3
None	100.0	100.0	100.0	100.0
3.6	106.0	96.0	104.6	105.6
7.2	106.1	95.2	103.7	104.4
14.4	97.9	100.0	102.3	106.8
28.8	92.4	97.6	104.4	107.4
57.5	88.5	96.8	107.8	107.6
115.0	73.4	96.0	94.2	108.9
230.0	67.3	92.2	90.6	110.1
460.0	57.6	86.5	81.7	108.3
920.1	40.6	72.4	75.9	103.8
1380.1	29.9	66.8	56.0	102.9
1840.1	24.1	62.8	54.0	101.2
2300.1	18.0	55.2	46.6	101.4
2760.2	18.0	51.6	46.0	94.7
3220.2	16.2	46.0	41.5	92.5
3680.3	16.1	42.8	41.5	88.6
4140.3	11.8	40.5	40.1	80.5
4600.3	11.5	33.2	37.9	67.1
5060.4	11.2	32.0	35.4	65.6

It may be seen that sodium chloride when added to the soil in the proportion of 7 parts of sodium per million of soil acts as a stimulant to the ammonifying organism of that soil. Sodium sulphate, on the other hand, even in the lowest concentration tested—3.6 parts per million of sodium—failed to stimulate the ammonifying organisms. Sodium nitrate stimulates in low concentration and has its greatest effect when 57.5 parts per million of sodium in the form of sodium nitrate is added. The carbonate has its greatest stimulating influence when 230 parts per million of sodium in the form of sodium carbonate is added to the soil. This compound continues to stimulate until the concentration exceeds 2,300 parts per million. The results, as a whole, indicate that it is the

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electro-negative ion which acts as the stimulant and not the sodium ion. The sodium chloride becomes toxic when only 14.4 parts per million of sodium as sodium chloride is added to the soil, while the sodium sulphate does not become toxic until twice this quantity is added. The nitrate does not become toxic until eight times as much has been added as is required of the chloride to retard ammonification, while it requires 190 times as much of the carbonate as of the chloride to retard ammonification.

These results confirm those obtained by C. B. Lipman (27) for the relative toxicity of the chloride, sulphate, and carbonate are in the order found by him. Furthermore, it shows that this order is held by these salts where applied to soils differing widely in composition. The results throughout are similar to Lipman's with the exception of the stimulation by sodium chloride in this series, and it is quite likely that

TABLE III

PERCENTAGE OF AMMONIA FORMED IN 100 GRAMS OF SOIL CONTAINING 2 GRAMS OF DRIED BLOOD AND VARYING AMOUNTS AND FORMS OF SODIUM SALTS IN EQUAL MOLECULAR PROPORTIONS

Fraction of Molecular Weight in 100 gm. of Soil	Per cent of Ammonia Formed in the Presence of			
	NaCl	Na_2SO_4	$NaNO_3$	Na_2CO_3
None	100.0	100.0	100.0	100.0
156×10^{-7}	106.0	95.2	104.6	104.4
312×10^{-7}	106.1	100.0	103.7	106.8
625×10^{-7}	97.9	97.6	102.3	107.4
125×10^{-6}	92.4	96.8	104.4	107.6
25×10^{-5}	88.5	96.0	107.8	108.9
5×10^{-4}	73.4	92.2	94.2	110.1
1×10^{-3}	67.3	86.5	90.6	108.3
2×10^{-3}	57.6	72.4	81.7	103.8
4×10^{-3}	40.6	62.8	75.9	101.2
6×10^{-3}	29.9	51.6	56.0	94.7
8×10^{-3}	24.1	42.8	54.0	88.6
10×10^{-3}	18.0	33.2	46.6	67.1

he would have observed this, even though working with soils widely different from those used in this work, had he used lower concentrations of the salt. We find 0.2 per cent the lowest concentration used by him, and this reduced the ammonifying powers to 30 per cent normal which is only slightly lower than the results obtained in this work when that concentration of sodium chloride was used. Furthermore, the results are in keeping for the nitrate obtained by Lipman and others.

The toxicity of the chloride increases much more rapidly than does the toxicity of the other compounds. The highest concentration tested, 5000 parts per million, nearly stopped ammonification where the chloride was added, but the sulphate had reduced it to only 32 per cent, the nitrate to 35.4 per cent, and the carbonate to only 65.6 per cent. The

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The results are reported in the form of the anion and not the cation monia produced in the untreated soil on the ammonifiers. The action of reporting the results makes the rate after the first stimulating instated as milligrams of ammonia throut all concentrations tested. however, materially change t equal molecular portions of the various monifying power of the unt own in Table III. gm. of soil. The results s ality of sodium chloride is much more toxic average of at least fo than is an equivalent molecular proportion of of closely agreeing de this in turn is more toxic than is an equivalent the comparative ir of sodium nitrate. Sodium carbonate is less toxic cation. salts tested. When $2x10^{-3}$ mol. of sodium chloride

TABLE IV

PERCENTA AMMONIA FORMED IN 100 GRAMS OF SOIL TO WHICH WERE 2 GRA GRAMS OF DRIED BLOOD AND VARYING AMOUNTS AND SC DIFFERENT FORMS OF POTASSIUM SALTS

Sodiu pe	Per cent of Ammonia Formed in the Presence of			
	KCl	$K_2(SO_4)$	KNO_3	K_2CO_3
—	100.0	100.0	100.0	100.0
6.1	94.6	99.6	102.2	103.8
12.2	95.8	98.4	100.4	105.8
24.4	91.9	98.4	101.6	107.7
48.9	89.1	98.9	99.1	108.0
97.8	85.9	90.9	98.3	106.1
195.5	80.5	87.0	95.0	106.1
391.0	76.3	86.0	89.5	106.1
782.0	64.2	74.1	83.5	107.2
1564.0	50.5	56.6	68.7	108.0
2346.0	42.7	53.7	50.7	105.8
3128.0	38.6	53.7	51.1	100.0
3910.0	27.9	51.8	55.4	98.1
4692.0	22.3	53.7	49.0	88.5
5474.0	22.3	51.5	41.6	89.2
6256.0	18.4	50.8	34.4	78.5
7038.0	15.6	41.8	30.5	75.8
7820.0	14.8	37.5	26.5	68.3
8602.0	14.8	30.7	25.8	54.6

is added to a soil, it reduces the soil's ammonifying power to 57.6 per cent. Equivalent quantities of sodium sulphate reduce it to 72.4 per cent, and of sodium nitrate to 81.7 per cent, while sodium carbonate in this proportion acts as a stimulant. In the highest concentrations of the salts tested, $10x10^{-3}$ mol., there was produced in the presence of sodium chloride 18.0 per cent of ammonia, while approximately twice as much ammonia was produced in the presence of the sulphate, three times as much in the presence of the nitrate, and four times as much in the presence of the carbonate.

Sodium chloride which has the lowest molecular weight is the most toxic of the sodium salts used, but the next in order of molecular weight is not the next in order of toxicity. However, the toxicity of the sodium salts varies with the electro-negative ion with which sodium is combined,

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e to a physiological influence exerted by it upon the protoplasm isms in addition to the osmotic effect.

Influence of Potassium Salts

nds used in the potassium series were the chloride, sul- e and carbonate. The concentrations were the same as d in the sodium series. The results as given in Table IV are rage of four or more closely agreeing determinations.

Neither potassium chloride nor potassium sulphate stimulates the ammonifiers in any of the concentrations tested. In this respect the potassium salts differ from the sodium salts, for the sodium chloride in the low concentrations acts as a stimulant. Potassium nitrate in the lowest concentrations stimulates, yet it is not as effective as is the corresponding sodium salt. Potassium carbonate exhibits its highest point of stimulation when 1500 parts per million of potassium in the form of this salt has been added to the soil.

TABLE V

PERCENTAGES OF AMMONIA FORMED IN 100 GRAMS OF SOIL CONTAINING 2 GRAMS OF DRIED BLOOD AND VARYING AMOUNTS AND FORMS OF POTASSIUM SALTS IN EQUAL MOLECULAR PROPORTIONS

Fraction of Molecular Weight in 100 gm. of Soil	Per cent of Ammonia Formed in the Presence of			
	KCl	K_2SO_4	KNO_3	K_2CO_3
None	100.0	100.0	100.0	100.0
156×10^{-7}	94.6	98.4	102.2	105.8
312×10^{-7}	95.8	98.4	100.4	107.7
625×10^{-7}	91.9	98.9	101.8	108.9
125×10^{-6}	89.1	90.9	99.1	106.1
25×10^{-5}	85.9	87.0	98.3	106.1
5×10^{-4}	80.5	86.0	95.0	106.1
1×10^{-3}	76.3	74.1	89.5	107.2
2×10^{-3}	64.2	56.6	83.5	108.0
4×10^{-3}	50.5	53.7	68.7	100.0
6×10^{-3}	42.7	53.7	50.7	88.5
8×10^{-3}	38.6	50.8	51.1	78.5
10×10^{-3}	27.9	37.5	55.4	68.3

Both potassium chloride and potassium sulphate are toxic in the lowest concentrations tested, and it is doubtful if even in lower concentrations they would act as stimulants. Potassium in the form of the nitrate does not become toxic until 48.9 parts per million have been added to the soil. The carbonate does not become toxic until nearly 4000 parts per million have been added to the soil. The toxicity of potassium in the form of the chloride and the sulphate rapidly increases with increasing concentrations until, when 1,564 parts per million have been added to the soil, the ammonifying power has been reduced to half its original value. The nitrate at this concentration has reduced it to 68.7 per cent, while the carbonate at this same concentration exerts its highest stimu-

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lating effect, or produces 108 per cent of ammonia. Above this concentration the toxicity of all the compounds increases in about the same order, so that when 8,602 parts per million of potassium in the form of the chloride has been added to the soil, it produces only 14.8 per cent as much ammonia as it does in the untreated soil. Twice as much is found in the soils containing the sulphate and nitrate, and three times as much in the soil receiving the carbonate. The results as a whole indicate that a greater effect is produced by the anion than by the cation.

The influence of equivalent molecular proportions of the various potasssium salts is given in Table V.

A given molecular quantity of potassium chloride is much more toxic to the ammonifiers than is an equivalent molecular quantity of the sulphate, nitrate, or carbonate, the toxicity of the compounds varying in the order named. If $4x10^{-3}$ mol. of potassium chloride or sulphate be added to 100 gm. of soil, the ammonifying power is reduced to about half normal. This quantity of carbonate is without effect. In the highest concentration of the salts used, $10x10^{-3}$, the chloride reduces the ammonifying powers to 27.9 per cent, the sulphate to 37.5 per cent, the nitrate to 55.4 per cent, and the carbonate to 68.3 per cent.

The toxicity of the potassium salts is governed by the electro-negative ion combined with the potassium, and where this is an ion of a strong acid the toxicity is much greater than where it is the ion of a weak acid, thus indicating that the osmotic pressure of the solution, while not the only factor, plays no small part in the observed action.

Influence of Calcium Salts

The compounds used in this series were calcium chloride, calcium nitrate, calcium sulphate and calcium carbonate. The first two were added to the soil according to the usual method, from a standard solution, while the sulphate and carbonate were weighed into the soil, carefully mixed, and then treated in the regular manner.

A number of determinations were made in each case and compared with sterile blanks, so that the results as reported in Table VI are the average of four or more closely agreeing determinations.

Neither the chloride nor the nitrate of calcium stimulated in any of the concentrations tested. The sulphate stimulates in all concentrations below 200 parts per million, while the carbonate, with the exception of the first two concentrations, stimulated in every case. This tends to indicate that even a calcareous soil, such as is being used in this work, may be stimulated in so far as its ammonifying powers are concerned by the addition of limestone. Furthermore, the stimulation is quite marked, for we find the addition of $2x10^{-3}$ mol. of calcium carbonate to 100 gm. of this soil raises its ammonifying powers to 112.2 per cent.

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Both the chloride and the nitrate are toxic in the lowest concentrations tested, while the sulphate does not become toxic until the concentration exceeds 400 parts per million. This would seem to indicate that its influence upon the physical properties of the soil must have something to do with its effect, for the soil solution would be saturated with the sulphate before this concentration was reached. After this first toxic effect, however, the ammonifying powers remained the same even where the largest quantities of calcium sulphate had been applied to the soil.

TABLE VI

PERCENTAGES OF AMMONIA FORMED IN 100 GRAMS OF SOIL TO WHICH WAS ADDED 2 GRAMS OF DRIED BLOOD AND VARYING AMOUNTS AND FORMS OF CALCIUM SALTS

Fraction of Molecular Wgt. in 100 gm. Soil	Calcium—Parts per Million	Per cent of Ammonia Formed in Presence of			
		$CaCl_2$	$CaSO_4$	$Ca(NO_3)_2$	$CaCO_3$
None	None	100.0	100.0	100.0	100.0
78×10^{-7}	3.13	95.7	100.3	91.2	97.3
156×10^{-7}	6.26	94.9	100.6	91.0	97.6
312×10^{-7}	12.52	87.6	102.6	90.0	101.2
625×10^{-7}	25.04	86.7	103.2	87.1	109.4
125×10^{-6}	50.08	83.0	103.5	86.2	108.4
25×10^{-6}	100.16	70.6	102.0	81.0	107.3
5×10^{-4}	200.32	59.7	103.2	74.6	108.7
1×10^{-3}	400.64	59.4	98.8	66.0	110.7
2×10^{-3}	801.28	41.8	98.0	76.2	112.2
3×10^{-3}	1201.92	38.5	98.6	75.8	111.5
4×10^{-3}	1602.56	34.2	98.8	68.0	109.7
5×10^{-3}	2003.20	32.1	91.0	60.4	110.4
6×10^{-3}	2403.84	32.1	93.6	62.7	108.7
7×10^{-3}	2804.48	25.4	97.4	61.8	108.7
8×10^{-3}	3205.12	24.2	95.7	51.8	114.6
9×10^{-3}	3605.76	23.3	96.0	37.8	104.8
10×10^{-3}	4006.40	19.7	95.1	27.9	104.8
11×10^{-3}	4407.04	19.1	96.5	22.3	107.6

In the presence of 4,407 parts per million of calcium in the form of the chloride the ammonia produced in the soil had been reduced to 19 per cent of the normal, while an equivalent quantity of the nitrate reduced it to 22 per cent. The sulphate at this concentration had reduced it only 3.5 per cent, while the carbonate was still stimulating. The influence of the calcium salt is therefore almost entirely governed by the electro-negative ion with which the calcium is combined.

It can be seen from these results that the addition of even 1 per cent of calcium carbonate to this soil, which already contains over 12 per cent of calcium carbonate, has a beneficial effect upon the ammonifying flora of the soil.

Furthermore, a given molecular quantity of calcium chloride is more toxic to the ammonifying organisms than is an equivalent molecular quantity of the nitrate, and this in turn is more toxic than the sulphate,

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and the sulphate more so than the carbonate. It may be noted that $2x10^{-3}$ mol. of calcium chloride reduces the ammonifying powers of the soil to less than one-half their original, while an equivalent quantity of the sulphate is only slightly toxic. The carbonate in this concentration acts as a stimulant. The calcium compounds when applied to a soil in molecular quantities are in the order of decreasing toxicity calcium chloride, calcium nitrate, calcium sulphate, and calcium carbonate.

Influence of Magnesium Salts

The compounds used in the magnesium series were the chloride, sulphate, nitrate and carbonate of magnesium. The last named was applied to the soil in the form of a dry powder, while all of the others were added in the usual manner. The results representing the average of four or more closely agreeing determinations are given in Table VII.

TABLE VII

PERCENTAGES OF AMMONIA FORMED IN 100 GRAMS OF SOIL CONTAINING 2 GRAMS OF DRIED BLOOD AND TO WHICH WAS ADDED VARYING AMOUNTS AND KINDS OF MAGNESIUM SALTS, THE UNTREATED SOIL BEING CONSIDERED AS PRODUCING 100 PER CENT

Fraction of Molecular Wgt. in 100 gm. Soil	Magnesium—Parts per Million in the Soil	Per cent of Ammonia Formed in Presence of			
		$MgCl_2$	$MgSO_4$	$Mg(NO_3)_2$	$MgCO_3$
None	None	100.0	100.0	100.0	100.0
$78x10^{-7}$	1.9	100.7	99.9	95.6	100.5
$156x10^{-7}$	3.8	99.0	98.7	90.7	101.6
$312x10^{-7}$	7.6	98.0	101.3	88.3	103.4
$625x10^{-7}$	15.2	96.6	104.5	82.3	103.5
$125x10^{-6}$	30.4	95.7	97.9	80.0	101.0
$25x10^{-5}$	60.8	95.6	94.5	92.0	101.0
$5x10^{-4}$	121.6	86.9	92.3	81.7	95.6
$1x10^{-3}$	243.2	74.9	84.1	83.0	91.3
$2x10^{-3}$	486.4	64.2	82.8	83.6	94.5
$3x10^{-3}$	729.6	64.2	82.8	75.1	89.8
$4x10^{-3}$	972.8	57.6	77.9	72.3	86.7
$5x10^{-3}$	1216.0	41.7	77.5	69.5	79.8
$6x10^{-3}$	1459.2	41.5	77.2	69.1	80.7
$7x10^{-3}$	1702.4	37.6	77.0	57.9	75.4
$8x10^{-3}$	1945.6	36.3	51.3	50.7	77.4
$9x10^{-3}$	2188.8	28.9	48.4	39.5	74.4
$10x10^{-3}$	2432.0	19.3	43.6	33.8	71.9
$11x10^{-3}$	2675.2	15.2	36.2	27.2	67.1

Both the sulphate and the carbonate of magnesium increase the ammonifying powers of the soil, both reaching their highest point of stimulation at about the same concentration. There is, however, this significant difference—the sulphate becomes toxic when slightly higher quantities are used, while the carbonate does not become toxic until 60.8 parts per million of magnesium have been added to the soil.

As in the calcium series neither the chloride nor the nitrate stimulate in any of the concentrations tested and the nitrate is quite toxic even in the lowest concentrations used. The toxicity of the chloride is just per-

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ceptible in the second concentration tested. The toxicity of the chloride does, however, increase more rapidly than does that of the nitrates, for when $5x10^{-3}$ mol. of magnesium chloride is added to 100 gm. of the soil, its ammonifying powers are reduced to 41.7 per cent, while an equivalent quantity of the nitrate reduces it to 69.5 per cent. At this same concentration the sulphate and carbonate reduce it to 77.5 per cent and 79.8 per cent, respectively. The largest quantity of the magnesium salts added, $11x10^{-3}$ mol. in 100 gm. of the soil, reduces the ammonia found in the case of the chloride to 15.2 per cent, the nitrate to 27.2 per cent, the sulphate to 36.2 per cent, and the carbonate to 67.1 per cent.

The depressing effect of the magnesium sulphate and carbonate upon the ammonifying flora is gradual and uniform, while that of the chloride, and especially that of the nitrate, are very irregular.

It would appear from these results that the addition of 0.4 per cent of magnesium to this soil has a deleterious effect upon the ammonifying organisms of the soil. This must not, however, be taken as the exact quantity which is toxic to ammonifying organisms, for it must be borne in mind that this soil normally carries over 8 per cent of magnesium carbonate.

Influence of Manganese Salts

The quantity of manganese present varies greatly in different soils and in some cases it is added as a soil amendment. Furthermore, many experiments have shown it to increase plant growth when applied to a soil, but very little has been done to determine its influence upon the bacterial activity of the soil. Therefore determinations were made of the ammonia produced in a soil to which varying quantities of manganous chloride, manganous sulphate, manganous nitrate and manganous carbonate had been added. The results so obtained as the average of a great number of closely agreeing determinations are given in Table VIII.

With the possible exception of the chloride, all of the manganese salts tested were strong stimulants to the ammonifying organisms of the soil. The extent of the stimulation and the quantity of the salt required to produce the maximum stimulation varies greatly with the salt used. Probably the chloride of manganese stimulates slightly in the very lowest concentration tested, while 17.2 parts per million of manganese in the form of the sulphate must be applied to produce the maximum stimulation. However, when this quantity is applied to the soil there accumulates in that soil one-fourth more ammonia than in the untreated soil. The concentration at which the nitrate reaches its maximum stimulation is the same as that for the sulphate, but the nitrate is not nearly as strong a stimulant as is the sulphate. The carbonate, with two exceptions, stimulates in all of the concentrations used, but its maximum stimulation is

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not reached until quantities much larger than are required of either the sulphate or the nitrate have been applied to the soil. While the carbonate stimulates throughout a much larger range of concentration than does any of the other manganese salts, it is not as powerful a stimulant as is the nitrate and especially the sulphate.

These results are intensely interesting when viewed in the light of results obtained by investigators who have studied the influence of manganese on higher plants. The majority of the investigators report the carbonate to be the greatest stimulant followed in the order, sulphate, nitrate and chloride. Furthermore, all workers have not found manganese compounds to stimulate the plants when applied directly to the soil, and the data herein reported offer a very plausible explanation of the lack of agreement among various reported experiments.

TABLE VIII

PERCENTAGES OF AMMONIA FORMED IN 100 GRAMS OF SOIL CONTAINING 2 GRAMS OF DRIED BLOOD TO WHICH WERE ADDED VARYING AMOUNTS AND KINDS OF MANGANOUS SALTS

Fraction of Molecular Wgt. in 100 gm. Soil	Added Parts per Million Manganese—	Per cent of Ammonia Formed in Presence of			
		$MnCl_2$	$MnSO_4$	$Mn(NO_3)_2$	$MnCO_3$
None	None	100.0	100.0	100.0	100.0
78×10^{-7}	4.3	100.6	112.0	108.2	101.3
156×10^{-7}	8.6	98.7	123.7	108.7	101.3
312×10^{-7}	17.2	100.0	123.8	116.0	106.9
625×10^{-7}	34.3	100.6	112.5	112.6	110.8
125×10^{-6}	68.6	91.3	98.4	108.2	108.7
25×10^{-6}	137.3	85.3	90.6	104.9	110.2
5×10^{-4}	274.6	82.9	85.6	93.9	111.2
1×10^{-3}	549.6	70.6	78.6	76.0	102.5
2×10^{-3}	1098.2	60.5	77.8	63.3	106.9
3×10^{-3}	1648.8	53.9	77.8	43.7	104.4
4×10^{-3}	2198.4	51.2	63.4	38.4	104.9
5×10^{-3}	2748.0	35.5	58.3	36.4	102.9
6×10^{-3}	3297.6	35.5	47.5	27.5	100.0
7×10^{-3}	3847.2	31.1	42.8	24.8	97.6
8×10^{-3}	4396.8	28.7	39.2	18.7	100.0
9×10^{-3}	4905.4	23.7	36.2	16.3	104.0
10×10^{-3}	5496.0	14.7	43.2	13.4	101.3
11×10^{-3}	6045.6	13.4	30.8	9.8	97.6

If we admit that much of the beneficial effect of the manganese on the plant is due to its stimulating influence on the bacterial activity of the soil, thus liberating more available nitrogen, forming organic acids and carbon dioxide which in turn liberate phosphorus and other elements essential to plant growth, we can readily see that its influence upon a plant growing in a soil well supplied with available nitrogen and phosphorus would not be great. But if the soil contained unavailable plant-food, the increased bacterial activity would make more plant-food available. This would then be taken up by the growing plant and shown in the increased crop yield.

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The chloride and the sulphate of manganese both became toxic when equivalent molecular proportions of the salts were applied to the soil, but four times as much of the nitrate as of the sulphate can be applied to a soil before it becomes toxic. The toxicity of the nitrate increases much more rapidly than does the toxicity of the chloride or sulphate. When $4x10^{-3}$ mol. of manganous chloride is applied to 100 gm. of soil, it reduces the ammonifying powers of the soil to 51.2 per cent, while an equivalent quantity of the sulphate reduces it to 63.4 per cent. The nitrate in this concentration reduces it to 38.4 per cent, but the carbonate is still acting as a stimulant. The highest concentration tested, $11x10^{-3}$ mol., practically stops ammonification in the presence of the nitrate and chloride, while the sulphate reduces it to one-third its original, but at this concentration the carbonate is without effect. In low concentrations the chloride is the most toxic of the salts tested while in higher concentrations the nitrate is the more toxic.

The Influence of Iron Salts

The compounds used in this series were ferric chloride, ferric sulphate, ferric nitrate and ferrous carbonate. All except the carbonate were added to the soil in solution. The carbonate was added in the form of a dry powder and carefully mixed with the soil. Considerable difficulty was experienced in getting duplicate determinations to agree when the sulphate was applied to the soil and the results as reported for sulphate represent the average of eight sets of determinations. The chloride, nitrate, and carbonate represent the average of four closely agreeing sets of determinations. The results are given in Table IX.

From these results it may be seen that all of the iron salts tested act as stimulants to the ammonifying organisms of the soil. The maximum stimulation for the chloride occurs when 2.9 parts per million of iron have been applied to the soil, while in the case of the other three compounds the greatest stimulation is not noticed until twice this quantity of iron in the various forms has been applied. Furthermore, the chloride is a much more powerful stimulant than is the sulphate, and in this respect there is a marked difference between the salt of manganese and that of iron.

The results offer a very likely explanation of why there is an increased yield obtained when iron compounds are applied to the soil, as the stimulation of the soil organisms would greatly increase the available plant-food. There would be not only more available nitrogen, but the increased bacterial activity would render soluble more potassium and especially more phosphorus; the results reported by Griffiths (15) indicate that the plants growing on soil manured with iron sulphate contain more phosphorus than do those growing on unmanured soil. We would have to assume either that the application of iron to the soil stimulates a plant

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so that it requires more phosphorus, or else that the iron compounds increase the availability of the phosphorus, and hence the plant takes up more. This latter explanation seems the more reasonable, but here we have to look for an indirect effect, for the iron directly depresses the solubility of phosphorus (14).

The ferric sulphate becomes toxic to the ammonifying organisms at a much lower concentration than do the other iron compounds, but it does not increase in toxicity nearly as rapidly as either the chloride or the nitrate. When 1489.2 parts per million of iron in the form of iron chloride or nitrate have been applied to a soil, its ammonifying powers are reduced to one-half their original, while an equivalent quantity of iron in the form of the sulphate reduces the ammonifying power to only 77.7 per cent. The carbonate, on the other hand, reduces it only 1.4 per cent.

TABLE IX

PERCENTAGES OF AMMONIA FORMED IN 100 GRAMS OF SOIL CONTAINING 2 GRAMS OF DRIED BLOOD AND TO WHICH HAD BEED ADDED VARYING AMOUNTS AND DIFFERENT FORMS OF IRON

Iron—Parts per Million Added	Per cent of Ammonia Formed in the Presence of			
	$FeCl_3$	$Fe_2(SO_4)_3$	$Fe(NO_3)_3$	$FeCO_3$
None	100.0	100.0	100.0	100.0
2.9	118.6	102.2	102.5	100.7
5.8	108.9	103.9	102.9	107.9
11.6	108.5	96.5	102.8	105.1
23.2	100.5	86.9	100.9	101.0
46.5	92.9	84.0	100.1	102.0
93.0	81.4	84.2	94.8	101.0
186.0	72.3	84.3	94.8	102.1
372.3	57.9	84.9	78.3	98.3
744.6	52.4	78.9	76.1	98.3
1116.9	52.1	79.7	67.1	99.0
1489.2	46.7	77.7	50.5	98.6
1861.5	40.6	73.9	44.5	96.3
2233.8	35.8	72.0	44.4	92.5
2606.1	36.2	78.7	44.5	93.5
2978.4	27.1	85.3	44.9	92.9
3350.7	25.7	72.3	44.1	92.2
3723.0	24.9	71.6	43.6	90.8
4095.3	17.7	76.8	30.3	87.8

The highest concentration used, 4095.3 parts per million of iron in the form of the chloride, reduces the ammonifying powers to 17.7 per cent, the nitrate to 30.3 per cent, the sulphate to 76.8 per cent, and the carbonate to 87.8 per cent.

The Influence of Chlorides

So far in this discussion we have been comparing the action of compounds having the same electro-positive but a varying electro-negative ion. Hence, the results considered have given us an insight into the influence of the anions Cl, SO_4, NO_3, and CO_3 upon the ammonifying efficiency of the soil. It is therefore important that the compounds be

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compared where the anion is a constant and the cation a variable. This is done in Table X. In this series we have the chlorides of sodium, potassium, magnesium calcium, manganese, and iron. The experiment was so arranged that equivalent quantities of chlorine in the various forms were applied to 100 gm. of soil. Each reported result is the average of four or more closely agreeing determinations.

TABLE X

PERCENTAGES OF AMMONIA FORMED IN 100 GRAMS OF SOIL CONTAINING 2 GRAMS OF DRIED BLOOD TO WHICH WAS ADDED VARYING AMOUNTS AND KINDS OF CHLORIDES, THE UNTREATED SOIL CONSIDERED AS PRODUCING 100 PER CENT

Cl—Parts per Million	Per cent of Ammonia Formed in the Presence of					
	NaCl	KCl	$MgCl_2$	$CaCl_2$	$MnCl_2$	Fe_2Cl_6
None	100.0	100.0	100.0	100.0	100.0	100.0
5.50	106.0	94.6	100.7	95.7	100.7	118.6
11.05	106.1	95.8	99.0	94.9	98.7	108.9
22.10	97.9	91.9	98.0	87.6	100.0	108.5
44.30	92.4	89.1	96.6	86.7	100.7	100.5
88.65	88.5	85.9	95.7	83.0	91.3	92.9
177.30	73.4	80.5	95.6	70.6	85.3	81.4
354.60	67.3	76.3	86.9	59.7	82.9	72.3
709.20	57.6	64.2	74.9	59.4	70.6	57.9
1418.40	40.6	50.5	64.2	41.8	60.5	52.4
2127.60	29.9	42.7	64.2	38.5	53.9	52.1
2836.80	24.1	38.6	57.6	34.2	51.2	46.7
3546.00	17.9	27.9	41.7	32.1	35.5	40.6
4255.20	17.9	22.3	41.5	32.1	35.5	35.8
4964.40	16.2	22.3	37.6	25.4	31.1	36.2
5673.60	16.1	18.4	36.3	24.2	28.8	27.1
6382.80	11.8	15.6	28.8	23.3	23.7	25.7
7092.00	11.5	14.8	19.3	19.7	14.7	24.9
7801.20	11.2	14.8	15.2	19.1	13.4	17.7

All of the chlorides tested, with two exceptions—calcium chloride and potassium chloride—increase the accumulation of ammonia in the soil, and it would appear from these results that the extent of stimulation is governed largely by the cation, while the toxicity of the compound is determined by the anion. Measured in terms of the effect upon ammonification, ferric chloride is the most effective stimulant of the chlorides tested, followed in the order of sodium chloride, magnesium chloride and manganous chloride. The last two stimulate only slightly. When 88.65 parts per million of chlorine in the form of iron or of manganese are applied to a soil they are toxic to the ammonifying organisms, while one-fourth this quantity of chlorine in the form of sodium is toxic and only one-half as much chlorine in the form of magnesium as in the form of sodium is necessary to decrease bacterial activity. Potassium chloride and magnesium chloride are toxic when only 5.5 parts per million are applied to a soil. If these two compounds act as stimulants it must be in very dilute solutions. Apparently the compounds, the cation of which has the highest atomic weight, are greater stimulants than those with

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lower atomic weights. Those compounds, which in dilute solution act as stimulants, in more concentrated solutions increase more rapidly in toxicity than do compounds which are not as active stimulants.

At the highest concentration tested, 7801.2 parts per million of chlorine, there is only 8 per cent more ammonia found in the presence of the least toxic than in the presence of the most toxic compound. However, at this high concentration the soil produces less than 20 per cent of the quantity of ammonia produced in untreated soil. The least is produced in the presence of sodium chloride and the greatest in the presence of calcium chloride. But these two compounds come very nearly together when they are compared from the viewpoint of equal molecular proportions of the various salts, as is done in Table XI.

TABLE XI

PERCENTAGES OF AMMONIA FORMED IN 100 GRAMS OF SOIL CONTAINING 2 GRAMS OF DRIED BLOOD AND TO WHICH WAS ADDED VARYING AMOUNTS AND KINDS OF CHLORIDES IN EQUAL MOLECULAR PROPORTIONS

Fraction of Molecular Wgt. in 100 gm. Soil	Per cent of Ammonia Formed in the Presence of				
	NaCl	KCl	$MgCl_2$	$CaCl_2$	$MnCl_2$
None	100.0	100.0	100.0	100.0	100.0
156×10^{-7}	106.0	94.6	98.8	94.9	98.7
312×10^{-7}	106.1	95.8	98.0	87.6	100.0
625×10^{-7}	97.9	91.8	96.6	86.7	100.6
125×10^{-6}	92.4	89.1	95.7	83.0	91.3
25×10^{-6}	88.5	85.9	95.6	70.6	85.3
5×10^{-4}	73.4	80.5	86.9	59.7	82.9
1×10^{-3}	67.3	76.3	74.9	59.4	70.6
2×10^{-3}	57.6	64.2	64.2	41.8	60.5
4×10^{-3}	40.6	50.5	57.6	34.2	51.2
6×10^{-3}	29.9	42.7	41.5	32.1	35.5
8×10^{-3}	24.1	38.6	36.3	24.2	28.7
10×10^{-3}	18.0	27.9	19.3	19.7	14.7

When 312×10^{-7} mol. of sodium chloride is applied to 100 gm. of soil it acts as a stimulant to the ammonifying organisms, but an equivalent quantity of any of the other salts tested is toxic. Were it not for the potassium salt which would have about the same osmotic pressure as the sodium, one might attribute the toxicity to the greater osmotic pressure exerted by the other salts; but the results show that while the increased osmotic pressure undoubtedly plays considerable part in retarding the ammonifying organisms; yet the nature of the salt also must be taken into account. With the single exception of sodium chloride the quantity of ammonia obtained is less when 156×10^{-7}mol. of the various salts are applied to the soil than when no salts are applied. At the highest concentration tested, 10×10^{-3} mol. per 100 gm. of soil, the potassium salt is least toxic, while the manganese salts are the most toxic. However, at this high concentration we find the quantity of ammonia produced in each very low and not a great difference between those receiving the various salts.

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Influence of Sulphates

The compounds used in this series were the sulphates of potassium, sodium, calcium, magnesium, manganese and iron. The quantity of the salt used in each case was such that equivalent quantities of SO_4 in the various forms were added to 100 gm. of soil. It was also of such a concentration, with the exception of the iron sulphate, that equal molecular proportions of the various salts were added to 100 gm. of soil. The results as averages of a number of closely agreeing determinations are given in Table XII.

TABLE XII

PERCENTAGES OF AMMONIA FORMED IN 100 GRAMS OF SOIL CONTAINING 2 GRAMS OF DRIED BLOOD AND TO WHICH WAS ADDED VARYING AMOUNTS AND FORMS OF SULPHATES, THE UNTREATED SOIL CONSIDERED AS PRODUCING 100 PER CENT

Fraction of Molecular Wgt. in 100 gm. Soil	SO_4— Parts per Million	Per cent of Ammonia Formed in the Presence of					
		Na_2SO_4	K_2SO_4	$CaSO_4$	$MgSO_4$	$MnSO_4$	$Fe_2(SO_4)_3$
None	None	100.0	100.0	100.0	100.0	100.0	100.0
78×10^{-7}	7.5	96.0	99.6	100.3	99.9	112.0	102.2
156×10^{-7}	15.0	95.2	98.4	100.6	98.7	123.7	103.9
312×10^{-7}	30.0	100.0	98.4	102.6	101.3	123.8	96.5
625×10^{-7}	60.0	97.6	98.9	103.2	104.3	112.5	86.9
125×10^{-6}	120.1	96.8	90.9	103.5	97.8	98.4	84.0
25×10^{-5}	240.2	96.0	87.0	102.0	94.4	90.6	84.2
5×10^{-4}	480.4	92.2	86.0	103.2	92.3	85.6	84.3
1×10^{-3}	960.8	86.5	74.1	98.8	84.0	78.6	84.9
2×10^{-3}	1921.5	72.4	56.6	98.0	82.8	77.8	78.9
3×10^{-3}	2882.3	66.8	53.7	98.6	82.8	77.8	79.7
4×10^{-3}	3843.1	62.8	53.7	98.8	77.9	63.4	77.7
5×10^{-3}	4803.8	55.2	51.8	91.0	77.3	58.3	73.9
6×10^{-3}	5764.6	51.6	53.7	93.6	77.2	47.5	72.0
7×10^{-3}	6725.5	46.0	51.5	97.4	70.7	42.8	78.7
8×10^{-3}	7686.1	42.8	50.8	95.7	51.3	39.2	85.3
9×10^{-3}	8647.0	40.5	41.8	96.0	48.4	36.2	72.3
10×10^{-3}	9607.7	33.2	37.5	95.1	43.6	34.2	71.6
11×10^{-3}	10568.6	32.0	30.7	96.5	36.2	30.8	76.8

Sodium sulphate and potassium sulphate are the only sulphates which fail to stimulate the ammonifying organisms. The intensity of the stimulation and the degree of stimulation varies with the different compounds. Calcium sulphate and manganous sulphate, which are so widely recognized as plant stimulants, have the greatest effect also upon the bacterial activity. It is quite likely that their influence upon the plant is only a secondary effect due to the action of the compound upon the soil organisms, which in turn liberate more plant-food. It is interesting to note that calcium sulphate, which is so widely known as a soil stimulant, increases the ammonia produced in the soil at first, but when 1×10^{-3} mol. or more is applied to 100 gm. of soil, it becomes slightly toxic. The toxicity, however, does not greatly increase as more of the salt is applied for we find 96.5 per cent of ammonia produced in the presence of 11×10^{-3} mol. The greater quantities of ammonia obtained in the lower concentrations of the salt cannot be attributed to the calcium sulphate

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preventing volatilization of the ammonia formed. Otherwise, we should expect the stimulation to increase or at least to remain the same where larger quantities of calcium sulphate are applied to the soil.

The toxicity of potassium sulphate is perceptible in the lowest concentration tested and gradually increases as the salt applied increases, until at $8x10^{-3}$ mol. the ammonifying power of the soil had been reduced to 50.8 per cent, and at the highest concentration tested, $11x10^{-3}$ mol. per 100 gm. of soil, it had been reduced to 30.7 per cent.

The influence of sodium sulphate on the ammonifying efficiency of the soils is almost identical with that of potassium sulphate, showing that the toxicity of the salt is governed largely by the electro-negative ion.

Magnesium sulphate and manganous sulphate differ from the sodium and potassium sulphates in that the former does not become toxic until $125x10^{-6}$ mol. of the salt has been applied to 100 gm. of the soil, but above this concentration the toxicity of these compounds increases more rapidly than does the toxicity of sodium sulphate or potassium sulphate. In the highest concentration tested, $11x10^{-3}$ mol., it is interesting to note that the toxicity of all the readily soluble salts is the same. From the reported data, it would appear that ferric sulphate is an exception to this statement, but it must be borne in mind that while the quantity of sulphate in this is the same as in the other sulphates yet the number of molecules of the salt is less. Furthermore, it would appear that the iron ion has actually had a protective influence against the toxic SO_4 ion. Moreover, ferric sulphate has a great deflocculating effect upon the soil, for after its addition the soil remains very light and porous.

The Influence of Nitrates

The compounds used in this series were sodium nitrate, potassium nitrate, calcium nitrate, magnesium nitrate, manganous nitrate and ferric nitrate. The quantity added to the soil was such that in each case equivalent quantities of NO_3 in the various forms were added to the soil. Hence, the varying factor is the electro-positive ion, the electro-negative remaining the same in each case. The average results for a number of closely agreeing determinations are given in Table XIII as percentages of ammonia formed in 100 gm. of soil, the untreated soil being taken as 100 per cent.

Sodium nitrate in the lower concentrations stimulates the ammonifying organisms of the soil and not until the quantity added reaches 310.4 parts per million of NO_3 does the compound become toxic. When 6208 parts per million have been added to the soil the ammonifying efficiency of the soil is reduced to less than half normal. At the highest concentration tested, 13,657.6 parts per million, the ammonia produced was still 35.4 per cent of its original.

Potassium nitrate is not as strong a stimulant as is the sodium nitrate and becomes toxic in lower concentration than does the sodium nitrate.

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However, the results as a whole show considerable similarity between the sodium and potassium compounds. At the highest concentration used potassium nitrate is considerably more toxic to ammonifying organisms than is sodium nitrate.

Calcium nitrate and magnesium nitrate are very similar in their action on the ammonifying powers of the soil. Neither stimulates in any of the concentrations tested, and they run nearly parallel throughout the entire range of concentration used. Calcium nitrate is slightly more toxic than is the magnesium nitrate. It is interesting to note the very small quantities of these substances required to depress the accumulation of ammonia in the soil. While they become toxic in very dilute solutions their toxicity does not increase as rapidly as does the toxicity of sodium nitrate and especially of potassium nitrate.

TABLE XIII

PERCENTAGES OF AMMONIA FORMED IN 100 GRAMS OF SOIL CONTAINING 2 GRAMS OF DRIED BLOOD TO WHICH WAS ADDED VARYING AMOUNTS AND FORMS OF NITRATE, THE UNTREATED SOIL CONSIDERED AS PRODUCING 100 PER CENT

NO_3—Parts per Million	$NaNO_3$	KNO_3	Per cent of Ammonia Formed in the Presence of			
			$Ca(NO_3)_2$	$Mg(NO_3)_2$	$Mn(NO_3)_2$	$Fe(NO_3)_3$
None	100.0	100.0	100.0	100.0	100.0	100.0
9.7	104.6	102.2	91.2	95.6	108.2	102.5
19.4	103.7	100.4	91.0	90.7	108.7	102.9
38.8	102.3	101.8	90.0	88.3	116.0	102.8
77.6	104.4	99.1	87.1	82.3	112.6	100.9
155.2	107.8	98.3	86.2	80.0	108.2	100.1
310.4	94.2	95.0	81.0	92.0	104.9	94.8
624.8	90.6	89.5	74.6	81.7	93.9	94.8
1241.6	81.7	83.5	66.0	83.0	76.0	78.3
2483.2	75.9	68.7	76.2	83.6	63.3	76.1
3724.8	56.0	50.7	75.8	75.1	43.7	67.1
4966.4	54.0	51.1	68.0	72.3	38.4	50.5
6208.0	46.6	55.4	60.4	69.5	36.4	44.5
7449.6	46.0	49.0	62.7	69.1	27.5	44.4
8691.2	41.5	41.6	61.8	57.9	24.8	44.5
9932.8	41.5	34.4	51.3	50.7	18.7	44.9
11174.4	40.1	30.5	37.8	39.5	16.3	44.1
12416.0	37.9	26.5	27.9	33.8	13.4	43.6
13657.6	35.4	25.8	22.3	27.2	9.8	30.3

Manganous nitrate when applied to soil in small quantities is a more powerful stimulant of the ammonifying organisms of the soil than are any of the other nitrates tested, but when used in large qauntities it is much more toxic than are any of the others. As a matter of fact, these results tend to bear out the general biological observation formulated by Hueppe that every substance, which in small quantities kills the protoplasm, in yet smaller quantities acts as a stimulant. Although manganous nitrate does not become toxic until 624.9 parts per million of the nitrate have been applied to the soil, in the highest concentration tested the ammonia produced is reduced to 9.8 per cent of normal, which is much lower than by any of the other nitrates.

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Ferric nitrate, while it increases the ammonifying powers of the soil, is not as efficient in this respect as is the manganous nitrate. It does, however, become toxic in slightly lower concentrations than the manganous nitrate. But the toxicity of the latter increases much more rapidly than does that of the former, for at the highest concentration tested we find three times the ammonia produced in the presence of ferric nitrate as at an equivalent concentration of the manganous nitrate, pointing to the conclusion that the toxicity of these compounds is governed by both anion and cation. It would be wrong, however, to conclude that the Mn ion is so much more toxic to the ammonifiers than is the Fe ion, for it must be borne in mind that more manganese than iron has been applied to the soil.

If we arrange the compounds in their decreasing order of toxicity as measured at the highest concentration when equivalent quantities of nitrates are added to a soil, they are manganous nitrate, potassium nitrate, magnesium nitrate, ferric nitrate and sodium nitrate. If we compare them when equivalent molecular proportions of the salts are added, using the highest concentration, $10x10^{-3}$ mol. per 100 gm. of soil, the order is changed and becomes manganous nitrate most toxic followed in the order named by ferric nitrate, calcium nitrate, magnesium nitrate, sodium nitrate and potassium nitrate.

The Influence of Carbonates

The compounds used in this series were the carbonates of sodium, potassium, calcium, magnesium, manganese and iron. The results as percentages of ammonia, considering the untreated soil as 100 per cent, are given in Table XIV.

Sodium carbonate stimulates the ammonifying organisms of this soil in all concentrations tested up to and including $5x10^{-3}$ mol. per 100 gm. of soil, while potassium carbonate stimulates in concentrations below this. Hence, the potassium salt is more toxic than is the sodium salt. Furthermore, the extent of stimulation in the case of sodium carbonate is greater than it is when potassium carbonate is added to the soil. The point at which the two reach their maximum stimulation is practically the same. Even in the highest concentrations, $11x10^{-3}$ mol. per 100 gm. of soil, large quantities of ammonia are produced.

Neither calcium carbonate nor manganous carbonate were found to be toxic in any of the concentrations tested, and the stimulating influence of both compounds is very marked. The soil to which $8x10^{-3}$ mol. of calcium carbonate was added produced 114.6 per cent of ammonia, while the maximum stimulating influence of the manganous carbonate is reached at a lower concentration, $5x10^{-4}$ mol.; although a strong stimulant, it seldom increases the ammonifying powers to as great an extent as does calcium carbonate. Considerable of the influence exerted by both of these compounds must be due to their improving the physical condition of the soil.

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For soils to which these are added never become hard and impervious to air and water as do soils to which potassium or sodium carbonate has been added.

The ferrous carbonate stimulates slightly until the quantity added exceeds $1x10^{-3}$ mol., but above this concentration, while it is toxic its toxicity never becomes great, most likely because of the comparative insolubility of the compound used.

TABLE XIV

PERCENTAGES OF AMMONIA FORMED IN 4 DAYS IN 100 GRAMS OF SOIL TO WHICH WAS ADDED 2 GRAMS OF DRIED BLOOD AND VARYING AMOUNTS AND DIFFERENT FORMS OF CARBONATES

Fraction of Molecular Wgt. in 100 gm. Soil	Million Parts per CO_3—	Per cent of Ammonia Formed in the Presence of					
		Na_2CO_3	K_2CO_3	$CaCO_3$	$MgCO_3$	$MnCO_3$	$FeCO_3$
None	None	100.0	100.0	100.0	100.0	100.0	100.0
$78x10^{-7}$	4.7	105.6	103.8	97.3	100.5	100.3	100.7
$156x10^{-7}$	9.4	104.4	105.8	97.6	101.6	101.3	107.9
$312x10^{-7}$	18.7	106.8	107.7	101.2	103.4	106.9	105.1
$625x10^{-7}$	37.5	107.6	108.0	109.4	103.5	110.8	101.0
$125x10^{-6}$	75.0	107.6	106.1	108.4	101.0	108.7	102.0
$25x10^{-5}$	150.0	108.9	106.1	107.3	101.0	110.2	101.0
$5x10^{-4}$	300.0	110.1	106.1	108.7	95.6	111.2	102.1
$1x10^{-3}$	600.0	108.3	107.2	110.7	91.3	102.5	98.3
$2x10^{-3}$	1200.0	103.8	108.0	112.2	94.5	106.9	98.3
$3x10^{-3}$	1800.0	102.9	105.8	111.5	89.8	104.4	99.0
$4x10^{-3}$	2400.0	101.2	100.0	109.7	86.7	104.9	98.6
$5x10^{-3}$	3000.0	101.4	98.1	110.4	79.8	102.9	96.3
$6x10^{-3}$	3600.0	94.7	88.5	108.7	80.7	100.6	92.5
$7x10^{-3}$	4200.0	92.5	89.2	108.7	75.4	97.6	93.5
$8x10^{-3}$	4800.0	88.6	78.5	114.6	77.4	100.0	92.9
$9x10^{-3}$	5400.0	80.5	75.8	104.8	77.4	104.0	92.2
$10x10^{-3}$	6000.0	67.1	68.3	104.8	71.9	101.3	90.8
$11x10^{-3}$	6600.0	65.6	54.6	107.6	67.1	97.6	87.8

In the presence of $10x10^{-3}$ mol. of the various compounds per 100 gm. of soil, the average amount of ammonia produced with the chloride is 18.32 per cent. In the presence of an equivalent quantity of the nitrates it is 35.42 per cent. With the sulphate it is 47.72 per cent, and in the presence of the carbonate it is 84.03 per cent. Thus measured in terms of their effect upon the amonifying efficiency of the soil, the chloride is the most toxic of the compounds tested, followed in order by nitrates, sulphates and carbonates.

Relationship between Ammonifying Organisms and Higher Plants

The results herein reported, together with those published by Dr. Harris (17) on "Effect of Alkali Salts in Soils on the Germination and Growth of Crops," make it possible to compare the influence of some of the salts upon the ammonifying powers of a soil with their influence upon the higher plants. This comparison is quite justifiable, for the same soil was used in the experiments with seedlings as has been used in this work on the ammonifiers. This comparison is made in Table XV.

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The results as reported are the quantities of the respective salts which are necessary to reduce ammonia produced, germination of wheat and production of dry matter in wheat to about half normal.

The chlorides of sodium, calcium, potassium and magnesium rank in the order named in toxicity to ammonifiers, but in the case of the plant, sodium nitrate is less toxic than is magnesium chloride. The quantities of the various chlorides which are necessary to reduce plant growth and ammonia to half normal were found to be very nearly the same. The order of toxicity of the salts, with the exception of sodium nitrate, sodium carbonate, magnesium nitrate and potassium sulphate is the same for both the wheat seedlings and the ammonifying organisms.

TABLE XV

PERCENTAGES OF VARIOUS SALTS IN LOAM SOIL WHICH ARE NECESSARY TO REDUCE AMMONIFICATION, GERMINATION AND DRY MATTER PRODUCED IN WHEAT TO ABOUT HALF NORMAL

	Ammonification to Half Normal	Wheat Seedling to Half Normal	Excess Required by Bacteria
Sodium Chloride	0.117	.20	.083
Calcium Chloride	0.222	.30	.078
Potassium Chloride	0.298	.25	.048
Magnesium Chloride	0.381	.40	.019
Potassium Nitrate	0.607	.40	.207
Sodium Nitrate	0.850	.30	.550
Sodium Sulphate	0.852	.55	.302
Magnesium Sulphate	0.963	.70	.263
Sodium Carbonate	1.166	.30	.866
Magnesium Nitrate	1.187	.45	.737
Potassium Sulphate	1.394	.60	.794
Potassium Carbonate	1.520	.70	.820

The other compounds are more toxic for the wheat seedlings than they are for ammonifying organisms. The greatest difference is in the case of sodium carbonate which was found to be very toxic to plants, but the bacteria withstand large quantities. Lipman (27) found ammonifying organisms to withstand even larger quantities than herein reported, for according to him it requires between 1.8 and 2 per cent of sodium carbonate to reduce the ammonia production to half normal.

Most of the salts tested are more toxic to wheat seedlings than they are toward the ammonifying organisms. This is very marked in the case of the carbonates, nitrates and sulphates, for it requires nearly twice the quantity of these to reduce ammonia produced to one-half normal as is required to reduce wheat seedling growth to the same extent.

The Relative Stimulation of the Various Salts

It has been noted repeatedly throughout this work that many of the salts tested stimulate the ammonifying organisms. The extent of stimulation and the concentration of the specific salts required to produce maximum stimulation vary greatly with the salt. These facts are summarized in figure 1.

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Only six of the compounds tested—calcium chloride, calcium nitrate, potassium chloride, potassium sulphate, magnesium nitrate and sodium sulphate—failed to stimulate in any of the concentrations. The other eighteen salts all stimulated the ammonifying organisms of the soil. Among these, however, there is a great variation in the extent of the stimulation. It is significant that we find as the more active stimulants those compounds which are most active in stimulating plant growth.

It is likely that the stimulation of the plant is due to a great extent to the increased available plant-food yielded by the increased bacterial activity. Averaging the molecular weights for the twelve compounds acting as the strongest stimulants, we find them to be slightly higher than the average molecular weight of those which exert little stimulating influence. But, comparing individual stimulants and non-stimulants we find no relationship to exist, for sodium chloride with the lowest molecular weight is a strong stimulant, while potassium sulphate with a high molecular weight is devoid of stimulating influence. Hence, it would seem that Grützner's generalization for animal stimulants will not hold for the ammonifying organisms of the soil.

The quantity of the salt necessary to produce maximum stimulation varies greatly with the salt. The iron compounds all stimulate most in low concentrations, while the carbonates of calcium, manganese and sodium have to be added in large quantities to a soil before maximum stimulation is noted. It is interesting to note that of the various salts studied by Miyake (41) and found by him to stimulate the rice plant, calcium chloride is the only one which stimulated the rice that does not stimulate the ammonifying organisms of the soil. Furthermore, Dr. Harris (17) found that sodium chloride, potassium sulphate, magnesium nitrate, calcium chloride and magnesium chloride failed to stimulate wheat seedlings, while sodium nitrate, sodium sulphate, magnesium sulphate, potassium nitrate, potassium chloride, potassium sulphate and sodium carbonate all acted as stimulants. Of these compounds sodium carbonate is the most active stimulant which is similar to the findings for the ammonifying efficiency of the soil.

Relative Toxicity of the Various Salts

The salts used in this work may be compared as to toxicity from three viewpoints: first, the lowest concentration of the salt at which a toxic effect is noted toward the ammonifying organisms; second, the molecular concentration at which ammonia produced is reduced to three-fourths normal; and third, the per cent of ammonia produced in the presence of the largest quantity of the various salts, which is 10×10^{-7} mol. of the salt in 100 gm. of soil. These results are given in figures 2, 3 and 4.

Five of the compounds tested—calcium nitrate, magnesium nitrate, calcium chloride, sodium sulphate and potassium sulphate—were toxic to

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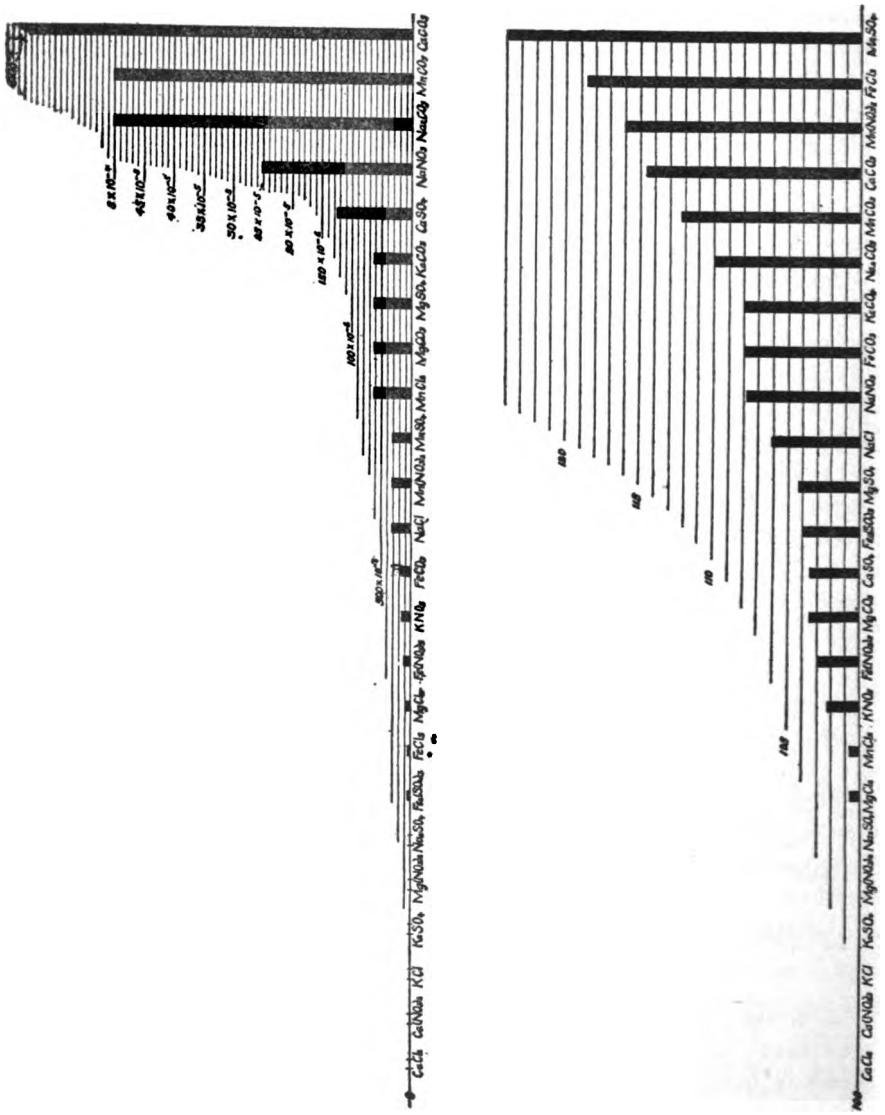

Fig. 1.—The upper graph represents the molecular concentrations at which the highest stimulation is noted.
The lower graph shows the percentage of stimulation at the above-noted molecular concentrations, the untreated cell being counted as producing 100 per cent of ammonia.

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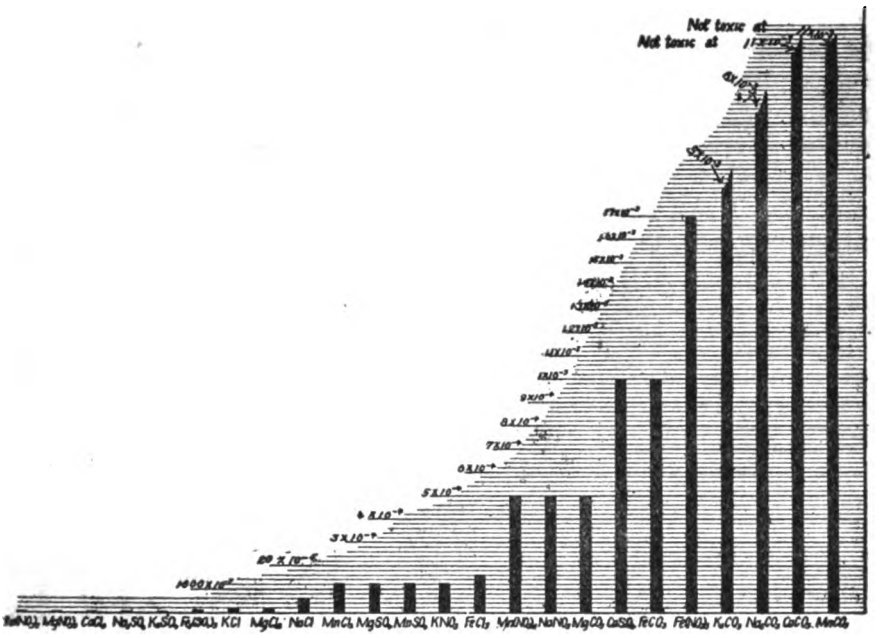

Fig. 2.—Diagram showing the molecular concentrations at which the various salts are toxic to ammonification.

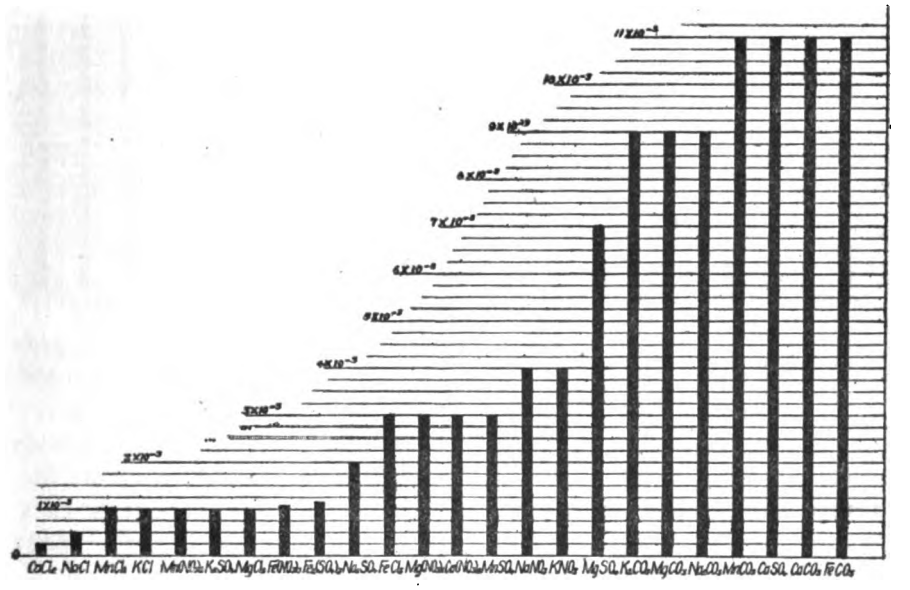

Fig. 3.—Diagram showing the molecular concentrations which reduce the ammonia produced to three-fourths normal.

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the ammonifiers in the lowest concentration tested, which was 78×10^{-3} mol. per 100 gm. of soil. All of the others, with the exception of two—calcium carbonate and manganous carbonate—became toxic in some of the concentrations tested. It is quite evident from these results that while the increased osmotic pressure exerted by the salts added to a soil plays an important part in the retarding of the bacterial activity, it is not the only nor probably the main influence. The main influence is likely to be a physiological one, due to the action of the substance upon the living protoplasm of the cell, changing its chemical and physical properties so that it cannot function normally. However, we do not find a relationship between the toxicity of the compound and its power to precipitate colloids. It appears, therefore, that while the precipitation of the collodial cellular material often causes death of the organisms, it is not necessarily the determining factor in the toxic action of these salts.

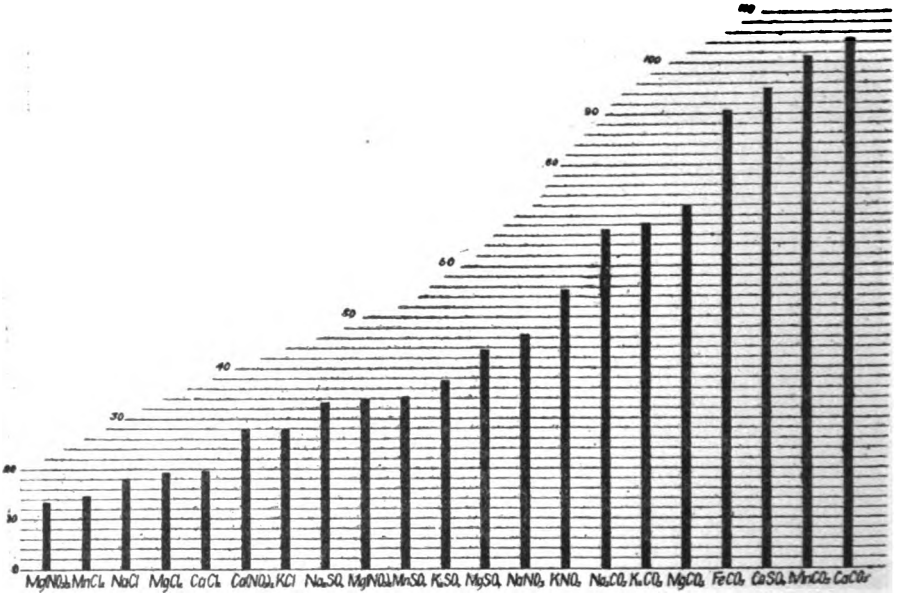

Fig. 4.—Diagram showing the percentages of ammonia produced in 100 gm. of soil to which had been added 10×10^{-3} molecules of the various salts. untreated soil being counted as producing 100 per cent.

As can be seen from figure 3 it is not necessarily those compounds which become toxic at the lowest concentration which have the most far-reaching effect upon the bacterial activity. For we find many of the compounds which were toxic at the lowest concentration tested must be added in fairly large quantities before the ammonia produced is reduced to three-fourths normal. For example, we find sodium sulphate toxic in 78×10^{-7} mol. concentration, yet it does not reduce the ammonia produced to three-fourths normal until 2×10^{-3} mol. concentration has been reached.

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While manganous chloride does not become toxic until 125×10^{-6} mol. has been added to 100 gm. of soil, yet at a concentration of 1×10^{-3} mol. it has reduced the ammonia production to three-fourths normal. From the results as a whole it can be seen that the common soil alkalies—sodium chloride, calcium chloride, sodium sulphate—and the less common one—calcium nitrate—are very toxic to ammonifying organisms, and if present to any great extent will greatly reduce the ammonia produced in the soil.

An examination of figure 4 shows conclusively that the toxicity of the compounds is controlled largely by the cation, for we find invariably at the concentration 10×10^{-3} mol. the chlorides and nitrates very greatly reduced the ammonifying powers of the soil, while the carbonates only slightly reduced the ammonia. The extent to which the carbonate reduces the ammonifying efficiency of the soil is nearly proportional to the solubility of the compound.

Summary

The toxicity of the chlorides, nitrates, sulphates, and carbonates of sodium, potassium, calcium, magnesium, manganese, and iron as determined by ammonification is controlled largely by the electro-negative ion and as a general rule, to which there are exceptions, the chlorides are the most toxic and nitrates, sulphates, and carbonates follow in the order of decreasing toxicity.

The quantity of a salt which can be applied to a soil without decreasing the ammonia formed varies with the salt and for the soil under investigation it is in the order of decreasing toxicity of the salts as follows: calcium chloride, calcium nitrate, sodium sulphate, potassium sulphate, magnesium nitrate, ferric sulphate, potassium chloride, magnesium chloride, sodium chloride, manganous chloride, magnesium sulphate, manganous sulphate, potassium nitrate, ferric chloride, manganous nitrate, sodium nitrate, magnesium carbonate, calcium sulphate, ferrous carbonate, ferric nitrate, potassium carbonate, sodium carbonate, manganous carbonate, and calcium carbonate. The last two were not toxic in any of the concentrations used.

It is not necessarily those compounds which become toxic in the lowest concentrations which are most toxic in higher concentrations; as the toxicity of some salts increases more rapidly than the toxicity of others.

It is quite evident from the results reported that the increased osmotic pressure exerted by the salt added to the soil plays a great part in the retarding of the bacterial activity but it is not the only factor. The main factor is probably a physiological one due to the action of the substance upon the living protoplasm of the cell changing its chemical and physical properties so that it cannot function normally.

The common soil "alkalies," sodium chloride, calcium chloride, sodium sulphate,—and the less common one—calcium nitrate—are very toxic to

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ammonifying organisms and if present in soil to any great extent will greatly reduce the ammonia produced in such a soil.

Calcium chloride, calcium nitrate, potassium chloride, potassium sulphate, magnesium nitrate, and sodium sulphate failed to increase the ammonia produced in a soil. All of the others, however, in some of the concentrations tested acted as stimulants. The extent of the stimulation and the quantity of salt necessary for maximum stimulation varied with the compound. Naming them in the order of increasing efficiency, they are: magnesium chloride, manganous chloride, potassium nitrate, ferric nitrate, magnesium carbonate, calcium sulphate, ferric sulphate magnesium sulphate, sodium chloride, sodium nitrate, ferrous carbonate, potassium carbonate, sodium carbonate, manganous carbonate, calcium carbonate, manganous nitrate, ferric chloride, and manganous sulphate.

Those compounds which are most active as stimulants to the higher plants are also most active in stimulating bacteria, and it is very likely that the effect upon the plant is due to the action of the compound upon the bacteria, which in turn render available more plant-food.

The quantity of sodium chloride, calcium chloride, potassium chloride and magnesium chloride required to reduce the ammonifying powers of the soil to half normal is practically the same as the quantity necessary to reduce the growth of wheat to the same extent. The ammonifying organisms are apparently more resistant to the other compounds tested than are the higher plants.

Literature Cited

(1) Allen, E. R., and Bonazzi, A.
1915. On nitrification: Preliminary observations. Ohio Agr. Exp. Sta. Tech. Bul. 7, p. 42.

(2) Beijerinck, M. W.
1913. Oxidation of manganous carbonate by microbes. *In* K. Akad. Wetensch. Amsterdam, Versl. Wis en Natuurk. Afdeel., Bd. 22, p. 415-420. Abs. *in* Exp. Sta. Rec., v. 32 (1915), p. 514.

(3) Brown, P. E.
1911. Some bacteriological effects of liming. Ia. Agr. Exp. Sta. Research Bul. 2, p. 49-107.

(4) Chester, F. D.
1901. Studies in soil bacteriology. *In* Del. Agr. Exp. Sta. 13th Ann. Rpt. p. 50-73.

(5) Chester, F. D.
1904. The bacteriological analysis of soils. Del. Agr. Exp. Sta. Bul. 65, p. 51-76.

(6) Dezani, S.
1911. Action of gypsum on nitrification. *In* Staz. Sper. Agr. Ital., v. 44, no. 2, p. 119-137. Abs. *in* Jour. Chem. Soc. [London], v. 100, pt. 2, p. 1019.

(7) Dumont, J.
1897. On the improvement of humus soils. Compt. Rend. Acad. Sci. [Paris], t. 125, p. 469-472.

Digitized by Google

(8) EBERMAYER, E.
1890. Untersuchungen über die Bedentung des Humus als Bodenbistandtheil und über des Einfluss des Waldes, Verschiedenen Bodenarten und Boden-decken auf die Zusammensetzung der Bodenluft. *In* Forschr. Agr. Phys., Bd. 13, p. 15-49.

(9) EHRENBERG, PAUL.
1904. Die bakterielle Bodenuntersuchung in ihrer Bedeutung für die Feststellung der Boden fruchtbarkeit. *In* Landw. Jahrb., Bd. 33, p. 139.

(10) ENGBERDING, D.
1909. Vergleichende Untersuchungen über die Bakterienzahl im Ackerboden in ihrer Abhangigkeit von ausseren Einflüssen. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 23, p. 569-642.

(11) FABRICIUS, O., and VON FEILITZEN, H.
1905. Ueber den Gehalt an Bakterien in jungfräulichem und Kultiviertem Hochmoorboden auf dem Versuchsfelds des Schwedischen Moorkulturvereins bei Flahult. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 14, p. 161-168.

(12) FISCHER, H.
1909. Ueber den Einfluss des Kalkes auf die Bakterien eines Bodens. *In* Landw. Vers. Stat., Bd. 70, p. 335.

(13) FRED, E. B., and HART, E. B.
1915. The comparative effect of phosphates and sulphates on soil bacteria. Wis. Agr. Exp. Sta. Research Bul. 35, 35 p.

(14) GREAVES, J. E.
1910. Effects of soluble salts on insoluble phosphates. *In* Jour. Biol. Chem., v. 7, p. 287-319.

(15) GRIFFITHS, A. B.
1889. A Treatise on Manures or the Philosophy of Manuring. Whittaker and Co., London.

(16) GUFFROY, C.
1907. The question of sulphate of iron. *In* Jour. Agr. Prat., n. s., t. 13, p. 782-786.

(17) HARRIS, F. S.
1915. Effect of alkali salts in soils on the germination and growth of crops. *In* Jour. Agr. Research, v. 5, p. 1-53.

(18) HARTWELL, B. L., and KELLOGG, J. W.
1905. On the effect of liming upon certain constituents of a soil. *In* R. I. Agr. Exp. Sta. 18th Ann. Rpt., p. 242-252.

(19) HILGARD, E. W.
1892. Ueber die Beziehung Zwischen Humus bildung und Kalkgehalt der Bodenarten. *In* Forschr. Agr. Phys., Bd. 15, p. 400-405.

(20) KELLERMAN, K. F., and ROBINSON, T. R.
1910. Lime and legume inoculation. *In* Science, v. 32, p. 159-160.

(21) KELLEY, W. P.
1912. The effects of calcium and magnesium carbonates on some biological transformations of nitrogen in soil. *In* Univ. Cal. Pub. Agr. Sci., v. 1, p. 39-49.

(22) KELLEY, W. P.
1915. Ammonification and nitrification in Hawaiian soils. Hawaii Agr. Exp. Sta. Bul. 37, p. 1-52.

Digitized by Google

(23) KRÜGER, —.
1908. Ein Beitrag zur Untersuchung der Stickstoffumsetzungen im Boden. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 23, p. 236.
(24) LEMMERMANN, O., and FRESENIUS, L.
1912. Ueber die Erhöhung der Ammoniak Bindenden Kraft des Bodens unter den Einfluss von Kohlensaurem Kalk. *In* Fühling's Landw. Ztg., Bd. 61, p. 240-253.
(25) LIPMAN, C. B.
1909. Toxic and antagonistic effects of salts as related to ammonification by Bacillus subtilis. *In* Bot. Gaz., v. 48, p. 105-125.
(26) LIPMAN, C. B.
1910. On the lack of antagonism between calcium vs. magnesium and also between calcium vs. sodium. *In* Bot. Gaz. v. 49, p. 41-50.
(27) LIPMAN, C. B.
1912. Toxic effects of "alkali salts" in soils on soil bacteria: I Ammonification. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 32, p. 58-64.
(28) LIPMAN, C. B.
1912. Toxic effects of "alkali salts" in soils on soil bacteria: II. Nitrification. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 33, p. 305-313.
(29) LIPMAN, C. B.
1913. Antagonism between anions as effecting ammonification in soils. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 36, p. 382-394.
(30) LIPMAN, C. B., and BURGESS, P. S.
1914. The effect of copper, zinc, iron and lead salts on ammonification and nitrification in soils. *In* Univ. Cal. Pub. Agr. Sci., v. 1, p. 127-139.
(30a) LIPMAN, C. B., and BURGESS, P. S.
1914. The protective action against magnesium carbonate of calcium carbonate for Azotobacter chroococcum. *In* Jour. Agr. Sci., v. 6, p. 484-498.
(31) LIPMAN, C. B., and SHARP, L. T.
1912. Toxic effects of "alkali salts" in soils on soil bacteria. III. Nitrogen fixation. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 35, p. 647-655.
(32) LIPMAN, J. G.
1902. Studies in nitrification. *In* Jour. Amer. Chem. Soc., v. 24, p. 171-186.
(33.) LIPMAN, J. G., BLAIR, A. W., OWEN, I. L., and MCLEAN, H. C.
1912. The availability of nitrogenous materials as measured by ammonification. N. J. Agr. Exp. Sta. Bul. 246, 36 p.
(34) LIPMAN, J. G., and BROWN, P. E.
1907. Ammonification in culture solution as affected by soil treatment. *In* N. J. Agr. Exp. Sta. 28th Ann. Rpt., p. 141-204.
(35) LIPMAN, J. G., BROWN, P. E., and OWEN, I. L.
1909. Experiments on ammonia and nitrate formation in soils. *In* N. J. Agr. Exp. Sta. 30th Ann. Rpt., p. 117-180.
(36) LIPMAN, J. G., BROWN, P. E., and OWEN, I. L.
1911. Experiments on ammonia and nitrate formation in soils, II. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 30, p. 156-181.
(37) LUMIA, C.
1914. The action of mineral fertilizers on the activity of certain soil bacteria. *In* Atti R. Accad. Lincei, Rend. Cl. Sci. Fis., Mat. e Nat. s. 5, v. 23, I, no. 9, p. 738-746. Abs. *in* Exp. Sta. Rec., v. 31, p. 821.

Digitized by Google

(38) LYON, T. L., and BIZZELL, J. A.
1909. Some conditions favoring nitrification in soils. *In* Science, n. s., v. 3, p. 773-774.

(39) MAKRINOV, I. A.
1908. Nitrification from the biological side. *In* Vyestnik Bakt. Aghron. Stantzii V. K. Ferrein, 1908, No. 14, p. 132-179. Abs. *in* Exp. Sta. Rec., v. 22, p. 318.

(40) MCBETH, I. G., and WRIGHT, R. C.
1912. Certain factors limiting nitrification. Abs. *in* Science, n. s. v. 35, p. 392. Also abs. *in* Exp. Sta. Rec., v. 26, p. 816.

(41) MIYAKE, K.
1914. The influence of salts common in alkali soils upon the growth of the rice plant. *In* Jour. Biol Chem., v. 16, p. 235-263.

(42) MONTANAN, C.
1914. Action of the oliodynamic elements on the nitrifying bacteria. *In* Staz. Sper. Agr. Ital., v. 47, no. 6, p. 441-448. Abs. *in* Exp. Sta. Rec., v. 33, p. 422.

(43) OLARU, M. D.
1915. Favorable action of mangenese on the bacteria of leguminous plants. *In* Compt. Rend. Acad. Sci. (Paris), t. 160, p. 280-283.

(44) OWEN, W. L.
1908. The effect of carbonates upon nitrification. Ga. Agr. Exp. Sta. Bul. 81, p. 1-42.

(45) PATERSON, J. W., and SCOTT, P. R.
1912. Influence of certain soil constituents upon nitrification. *In* Jour. Dept. Agr. Victoria, v. 10, p. 393-400.

(46) PECK, S. S.
1911. Some biochemical investigations of Hawaiian soils, with special reference to fertilizing with molasses. Hawaiian Sugar Planters' Sta. Div. Agr. and Chem. Bul. 34, p. 1-39.

(47) PETERSON, P.
1871. Ueber den Einfluss des Mergels auf die Bildung von Kohlensäure und Salpetersaure im Ackerboden. *In* Landw. Vers. Stat., Bd. 13, p. 160.

(48) PICHARD, P.
1884. Action nitrifiante comparée de quelques contenus naturellement ou ajoutés dans les terres végétales. *In* Compt. Rend. Acad. Sci. [Paris], t. 98, p. 1289-1290.

(49) REMY, T. H.
1902. Bodenbakteriologische Studien. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 8, p. 657-662.

(50) RENAULT, P.
1910. Potash and the mobilization of organic nitrogen in humus soils. *In* Engrais, t. 25, no. 5, p. 132-135. Abs. *in* Exp. Sta. Rec., v. 22, p. 714.

(51) SCHLÖSING, —.
Cited by Warington, R., *in* "Six lectures on the investigations at Rothamsted Experimental Station. U. S. Dept. Agr. Off. Exp. Sta. Bul. 8, p. 94.

(52) SEWERIN, S. A.
1904. Gips als ammoniakbindende Substunz bei der Verrottung des Stallmistes. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 11, p. 389-396, 442-451.

Digitized by Google

(53) SKINNER, J. J., SULLIVAN, M. X. et al.
1914. The action of manganese in soils. U. S. Dept. Agr. Bul. 42, 42 p.
(54) VOORHEES, E. B., and LIPMAN, J. G.
1908. Some chemical and bacteriological effects of liming. *In* N. J. Agr. Exp. Sta. Bul. 210, 79 p. (also N. J. Agr. Exp. Sta. Ann. Rpts. for 1906, 1907 and 1908).
(55) WARINGTON, ROBERT.
1884. On nitrification: Part III. *In* Trans. Chem. Soc. London, v. 45, p. 637-672.
(56) WITHERS, W. H., and FRAPS, G. S.
1900. The relative value of some nitrogenous fertilizers. *In* N. C. Agr. Exp. Sta. Bul. 176, p. 15-22.
(57) WOLLNY, G.
1886. Untersuchungen über die Bersetzung der organischen Substanzen. *In* Jour. Landw., Bd. 34, p. 213-325.

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ON THE NATURE OF AMMONIFICATION AND NITRIFICATION[1]

By

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It has been found by many experiments on ammonification and nitrification that the rates of increase of ammonia and nitric acid produced at first rise very slowly, then very rapidly and later slowly again. If the rates are put in the form of a curve, the curve is very similar to that of an autocatalytic chemical reaction in which one of the products of reaction accelerates the reaction. It will be of interest, therefore, to study the nature of these microbiological chemical phenomena from the standpoint of autocatalysis.

THE QUANTITATIVE RELATION BETWEEN THE AMOUNT OF REACTION-PRODUCTS AND THE TIME OF AUTOCATALYSIS

The differential equation which is characteristic of the initial stages of autocatalytic mono-molecular reaction is as follows:

$$\frac{dx}{dt} = k_1 x (a - x) \quad \ldots\ldots (1)$$

which expresses in mathematical symbols the fact that the velocity of the transformation is, at any instant, proportional to the amount of material which is undergoing change and to the amount of material which has already been transformed. If, however, the reaction has proceeded so far that the depressant effect of the products of the reaction upon its velocity is measurable, equation (1) becomes:

$$\frac{dx}{dt} = k_1 x (a - x) - k_2 x^2 \quad \ldots\ldots (2)$$

This equation can, however, be reduced to the same form as (1), only the constants have a somewhat different meaning, for equation (2) is obviously the same as the following equation:

$$\frac{dx}{dt} = (k_1 + k_2) x \left(\frac{k_1}{k_1 + k_2} a - x\right) \quad \ldots\ldots (3)$$

which is of the same form as equation (1) but instead of k_1 we have $k_1 + k_2$, and instead of a we have $\frac{k_1}{k_1 + k_2} a$.

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Equation (3) may therefore be written:

$$\frac{dx}{dt} = kx\,(A - x) \quad \ldots\ldots (4)$$

where k and A are constants.

Integrating equation (4), we obtain:

$$\log \frac{x}{A - x} = Akt + C \quad \ldots\ldots (5)$$

where C is the constant of integration.

Now, it is evident that when $x = \frac{1}{2} A$, that is, when the reaction has proceeded half way to equilibrium, $\log \frac{x}{A - x} = 0$. Choosing C, therefore, so as to make the right-hand side of equation (5) vanish at the same time as the left-hand side we have:

$$\log \frac{x}{A - x} = Ak\,(t - t_1) \quad \ldots\ldots (6)$$

where t_1 is the time at which the reaction is half completed. This equation may obviously be written,

$$\log \frac{x}{A - x} = K\,(t - t_1) \quad \ldots\ldots (7)$$

If an increase of reaction products with time can be regarded as expressing, primarily, the progress of an autocatalytic reaction, equation (7) should hold good. Where x represents the amount of reaction product at the time t, and A represents the maximum or final amount of reaction product, t_1 is the time at which half this maximum amount has been attained, and K is a constant which must be determined from a known value of x at a given time t.

On differentiating equation (4) with respect to x, calling $-\frac{dx}{dt} = v$ we obtain:

$$\frac{dv}{dx} = k\,(A - 2x) \quad \ldots\ldots (8)$$

Differentiating again we obtain:

$$\frac{d^2v}{dx^2} = -2k \quad \ldots\ldots (9)$$

The right-hand side of equation (8) vanishes and $\frac{d^2v}{dx^2}$ is negative

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when $x = \frac{A}{2}$, hence $\frac{dx}{dt}$ is at a maximum when $x = \frac{A}{2}$. In other words the rate of increase of reaction products during a given period of reaction should be at a maximum when the reaction of that period is half completed.

Application to Ammonification

I.

Lipman (2), working in collaboration with Blair, Owen and McLean, has carried out a series of determinations upon the daily increase of ammonia formed in ammonification. In his experiment, 80 gm. of soil, 20 gm. of sand, 0.5 gm. of calcium carbonate and dried blood equivalent to 465 mg. of nitrogen were used. Six c.c. of soil infusion, 12 c.c. of water and 60 per cent of moisture for organic matter were added. The mixture was kept in tumblers at room temperature. The amounts of ammonia nitrogen recovered are indicated in the following table and the accompanying curve. On examining the table in which the daily increase of ammonia nitrogen is indicated, choosing the points with which to determine the constants of the curve sufficiently far apart and in such a manner that each pair of points on the curve is separated by an interval of 2 days, we find that:

When time = 2 days, Weight of ammonia nitrogen = 8.11 mg.
When time = 4 days, Weight of ammonia nitrogen = 46.03 mg.
When time = 8 days, Weight of ammoina nitrogen = 164.87 mg.
When time = 10 days, Weight of ammonia nitrogen = 185.91 mg.

Applying equation (7) to these values we find:

$$\log \frac{8.11}{A - 8.11} = K(2 - t_1) \quad \ldots\ldots\ldots\ldots\ldots\ldots (10)$$

$$\log \frac{46.03}{A - 46.03} = K(4 - t_1) \quad \ldots\ldots\ldots\ldots\ldots\ldots (11)$$

$$\log \frac{164.17}{A - 164.17} = K(8 - t_1) \quad \ldots\ldots\ldots\ldots\ldots\ldots (12)$$

$$\log \frac{185.91}{A - 185.91} = K(10 - t_1) \quad \ldots\ldots\ldots\ldots\ldots\ldots (13)$$

From (10) and (11), by subtraction we obtain:

$$\log \frac{46.03\,(A - 8.11)}{8.11\,(A - 46.03)} = 2K \quad \ldots\ldots\ldots\ldots\ldots\ldots (14)$$

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While from (12) and (13) we obtain:

$$\log \frac{185.91\ (A - 164.17)}{164.17\ (A - 185.91)} = 2K \quad \ldots\ldots\ldots\ldots\ldots\ldots (15)$$

Hence, from (14) and (15) we have:

$$\frac{46.03\ (A - 8.11)}{8.11\ (A - 46.03)} = \frac{185.91\ (A - 164.17)}{164.17\ (A - 185.91)} \quad \ldots\ldots (16)$$

Solving this equation for A we find:

$$A = 189.83$$

A convenient check upon the above calculation is afforded by the fact expressed in equations (8) and (9), namely that the rate of increase in x, that is, daily increase in weight, should be at a maximum when x (= amount of ammonia nitrogen) $= \frac{1}{2} A$, in this case 94.94 mg.

Examining the table we find that:

At time = 3 days, Weight of ammonia nitrogen = 19.80 mg.
Daily increase = 11.70 mg.

At time = 4 days, Weight of ammonia nitrogen = 46.03 mg.
Daily increase = 26.23 mg.

At time = 5 days, Weight of ammonia nitrogen = 68.66 mg.
Daily increase = 22.63 mg.

At time = 6 days, Weight of ammonia nitrogen = 123.23 mg.
Daily increase = 54.57 mg.

At time = 7 days, Weight of ammonia nitrogen = 144.27 mg.
Daily increase = 21.04 mg.

At time = 8 days, Weight of ammonia nitrogen = 164.87 mg.
Daily increase = 20.60 mg.

Thus we see that when the amount of ammonia nitrogen is between 68.66 and 123.23 mg., the rate of increase in ammonia nitrogen is at a maximum. Hence we may conclude that the value of A deduced from equation (16) is the correct one.

Now we find from the curve that the amount of ammonia nitrogen is 94.94 mg. at 5.6 days. Hence equation (7) becomes for this case:

$$\log \frac{x}{189.83 - x} = K\ (t - 5.6) \quad \ldots\ldots\ldots\ldots\ldots\ldots (17)$$

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Now, when $t = 1$ we have:

$$\log \frac{5.56}{184.27} = -4.6K$$

$$K = 0.3305$$

Similarly:

$t = 2$	$K = 0.3753$
$t = 4$	$K = 0.3100$
$t = 5$	$K = 0.4097$
$t = 8$	$K = 0.3415$
$t = 10$	$K = 0.3810$

The average value of K from these values is 0.358, hence equation (7) becomes:

$$\log \frac{x}{189.83 - x} = 0.358\,(t - 5.6) \quad \ldots\ldots\ldots\ldots (18)$$

By giving t the successive value 1, 2, 3, etc., we can calculate the corresponding theoretical value of x, that is, of the weight of ammonia nitrogen. In Table I and figure 1 the theoretical and experimental results are compared.

TABLE I

COMPARISON OF AMOUNT OF AMMONIA NITROGEN FOUND WITH THAT CALCULATED BY FORMULA

Time, Days	Ammonia Nitrogen mg.	
	Found	Calculated
1	5.55	4.19
2	8.11	9.29
3	19.80	19.94
4	46.03	40.10
5	68.66	72.01
6	123.23	110.55
7	144.27	144.78
8	164.87	166.99
9	172.70	179.23
10	185.91	185.20

It will be seen that in nearly every case the observed and calculated values agree well.

II

Applying the same method to another observation of Lipman's upon the daily increase of ammonia nitrogen in ammonification in which dried blood equivalent to 310 mg. of nitrogen was added, and using the same points ($t = 2, 4, 8$ and 10) on the curve to determine A, we find:

$$A = 133.13$$

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Hence $\frac{1}{2}A = 66.57$. Now referring to Table II we find that:

At time = 3 days, Weight of ammonia nitrogen = 15.14 mg.
Daily increase = 9.06 mg.

At time = 4 days, Weight of ammonia nitrogen = 31.07 mg.
Daily increase = 15.93 mg.

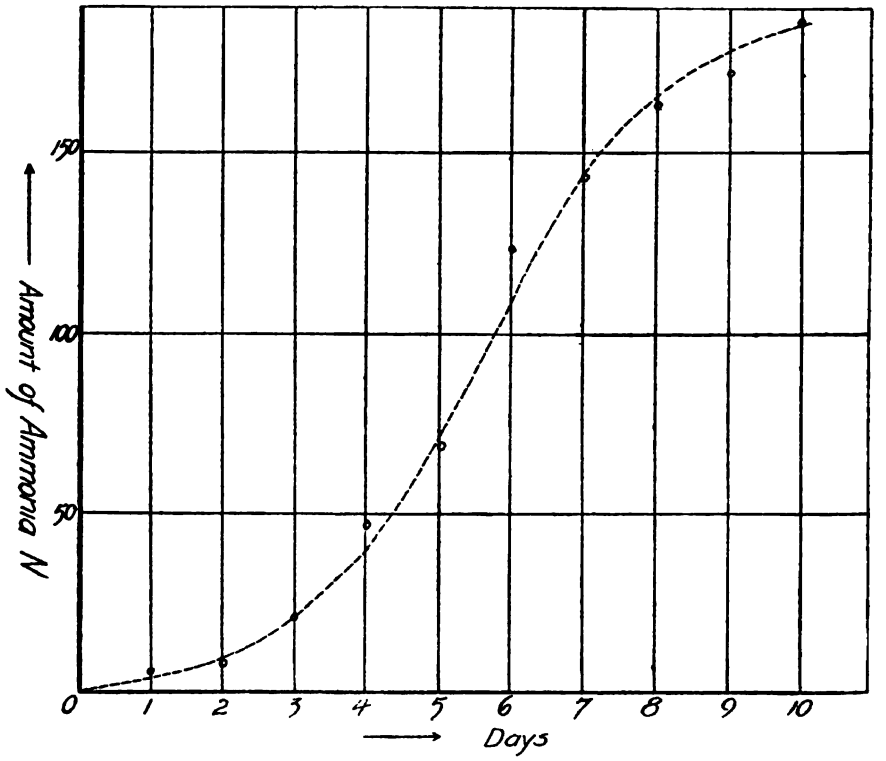

Fig. 1.—Diagram showing a comparison of the amount of ammonia nitrogen found with that calculated by formula.

At time = 5 days, Weight of ammonia nitrogen = 51.14 mg.
Daily increase = 20.07 mg.

At times = 6 days, Weight of ammonia nitrogen = 81.07 mg.
Daily increase = 29.93 mg.

At time = 7 days, Weight of ammonia nitrogen = 98.32 mg.
Daily increase = 17.25 mg.

At time = 8 days, Weight of ammonia nitrogen = 108.27 mg.
Daily increase = 9.95 mg.

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Hence the agreement between the theoretical and actual curves of increase of ammonia nitrogen is very good, for when $x = \frac{1}{2}A = 66.57$ the daily increment has reached its maximum.

On the curve we find 5.6 days corresponding to 66.57 mg. of ammonia nitrogen. Hence the equation becomes for this case:

$$\log \frac{x}{133.13 - x} = K\,(t - 5.6)$$

From all of the observed value we find that the average value of K is 0.3352, hence the equation becomes:

$$\log \frac{x}{133.13 - x} = 0.3352\,(t - 5.6)$$

As before, giving the successive value 1, 2, 3, etc. for t, we can compute the corresponding theoretical value of x. In Table II the theoretical and observed results are compared.

TABLE II

COMPARISON OF AMOUNT OF AMMONIA NITROGEN FOUND WITH THAT CALCULATED BY FORMULA

Time, Days	Ammonia Nitrogen mg.	
	Found	Calculated
1	4.24	3.72
2	6.08	7.78
3	15.14	15.77
4	31.07	30.00
5	51.14	51.41
6	81.07	76.75
7	98.32	99.39
8	108.27	115.06
9	119.36	124.14
10	128.51	128.78

III

Applying the same method to another experiment of Lipman's in which dried blood equivalent to 155 mg. of nitrogen was added and using the points ($t = 3, 5, 6$ and 8) on the curve to determine A, we find:

$$A = 82.10$$

$$\frac{A}{2} = 41.05$$

The daily increment of ammonia nitrogen is at a maximum between 25.52 and 54.04 mg. of ammonia nitrogen. Hence the value of A deduced from the equation is the correct one. The time to produce 41.05

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mg. of ammonia nitrogen is 5.6 days. Hence the equation becomes for this case:

$$\log \frac{x}{82.10 - x} = K\,(t - 5.6)$$

By calculating the value of K from the observations at $t = 1, 2, 3, 4, 7$ and 8 we find, respectively, 0.2927, 0.3461, 0.3698, 0.4134, 0.3889 and 0.3781. The average of these values is 0.3651. Hence the equation becomes:

$$\log \frac{x}{82.10 - x} = 0.3651\,(t - 5.6)$$

TABLE III

COMPARISON OF AMOUNT OF AMMONIA NITROGEN FOUND WITH THAT CALCULATED BY FORMULA

Time, Days	Ammonia Nitrogen mg.	
	Found	Calculated
1	3.34	1.68
2	4.41	3.79
3	8.13	8.21
4	14.70	16.96
5	25.52	30.91
6	54.04	47.90
7	63.73	62.76
8	73.06	72.44
9	55.10	77.10
10	61.53	80.10

Now giving t the successive value 1, 2, 3, etc., we find the following theoretical value of x.

The discrepancy in the last two values is evidently due to the accompanying nitrification in which ammonia formed undergoes change to nitric acid.

Application to Nitrification

I

Warington (3) has made an experiment upon the growth of nitric acid in nitrification. By the application of the method above described to his result we find:

$$\log \frac{1.1}{A - 1.1} = K\,(62 - t_1)$$

$$\log \frac{9.6}{A - 9.6} = K\,(77 - t_1)$$

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$$\log \frac{13.1}{A - 13.1} = K\,(81 - t_1)$$

$$\log \frac{18.7}{A - 18.7} = K\,(96 - t_1)$$

$$\frac{9.6\,(A - 1.1)}{1.1\,(A - 9.6)} = 15\,K$$

$$\frac{18.7\,(A - 13.1)}{13.1\,(A - 18.7)} = 15\,K$$

$$\frac{9.6\,(A - 1.1)}{1.1\,(A - 9.6)} = \frac{18.7\,(A - 13.1)}{13.1\,(A - 18.7)}$$

$$A = 19.2$$

$$\frac{A}{2} = 9.6$$

$$t_1 = 77.$$

Then the equation becomes:

$$\log \frac{x}{19.2 - x} = K\,(t - 77)$$

By calculation of K from the observations at $t = 62$, 81 and 96 we have, respectively, 0.0811, 0.0830 and 0.0828. The mean of these values is 0.0823. Hence the equation becomes:

$$\log \frac{x}{19.2 - x} = 0.0823\,(t - 77)$$

Now giving t the value of 62, 77, 81 and 96 we can calculate their corresponding theoretical value of x, as will be seen in Table IV in which the observed and calculated values of x are compared.

TABLE IV

COMPARISON OF AMOUNT OF NITROGEN AS NITRIC ACID FOUND WITH THAT CALCULATED BY FORMULA

Time, Days	N as HNO_3 per million	
	Found	Calculated
62	1.1	1.06
(77	9.6	9.60)
81	13.1	13.08
96	18.7	18.69

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Owing to the fact that the experiment was made at intervals too infrequent to enable us to determine A, the number in parenthesis, therefore, was measured by the author from the curve and added.

The agreement between the observed and calculated values is quite good.

II

Applying the same method to another result of nitrification by Warington we find also that the theoretical values of x nearly coincide with those of observation. Using the points ($t = 22$, 35, 52 and 65) on the curve to determine A, we have:

$$A = 25.3$$

$$\frac{A}{2} = 12.7$$

$$t_1 = 50.$$

Then the equation becomes:

$$\log \frac{x}{25.3 - x} = K\ (t - 50)$$

Giving t all of the observed values the average value of K is calculated to be 0.0501. Hence the equation becomes:

$$\log \frac{x}{25.3 - x} = 0.0501\ (t - 50)$$

Now giving t the values of 22, 35, 49, 52 and 65 we find that the corresponding theoretical values of x are 0.96, 3.81, 11.92, 14.10 and 21.49, respectively. In Table V the observed and calculated values are compared:

TABLE V

COMPARISON OF AMOUNT OF NITROGEN AS NITRIC ACID FOUND WITH THAT CALCULATED BY FORMULA

Time, Days	N as HNO_3 per million	
	Found	Calculated
22	0.7	0.96
35	2.0	3.81
49	11.9	11.92
(52	14.2	14.10)
65	20.1	21.49

III

Applying the same method to one other experiment on nitrification obtained by Lemmermann, Fischer and Heintz (1), we find good agree-

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ment between the values of observation and calculation. Using the points ($t = 0$, 14, 28 and 42) on the curve for the determination of A we have:

$$A = 15.53$$
$$\frac{A}{2} = 7.77$$
$$t_1 = 13.$$

Assuming that the values obtained are correct, the equation becomes:

$$\log \frac{x}{15.53 - x} = K\,(t - 13)$$

Now when $t = 0$ we have:

$$\log \frac{4.70}{10.83} = -13\,K$$

whence $K = 0.0279$, similarly from the observations at $t = 14$, 28, 42 and 49 we find, respectively, $K = 0.0236$, 0.0353, 0.0316 and 0.0345. The average value of K from these observations is 0.0305, hence the equation becomes:

$$\log \frac{x}{15.53 - x} = 0.0305\,(t - 13)$$

Now giving t the values of 0, 14, 28, 42, 49 and 77, we can compute the corresponding theoretical value of x. In Table VI the theoretical and experimental values are compared.

TABLE VI

COMPARISON OF AMOUNT OF NITRATE NITROGEN FOUND WITH THAT CALCULATED BY FORMULA

Time, Days	Nitrate Nitrogen mg.	
	Found	Calculated
0	4.70	4.45
14	7.97	8.03
28	11.99	11.51
(42	13.85	13.74)
49	14.69	14.30
77	14.26	15.35

The agreement between calculated and actual values of nitrate nitrogen is as perfect as in the previous cases.

SUMMARY

The preceding discussion may be summarized as follows:

1. The processes of ammonification and nitrification are autocatalytic chemical reactions.

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2. In ammonification and nitrification the maximum increase of ammonia and nitric acid in a unit of time occurs when the total amount of production due to these processes is half completed.

3. Increases of ammonia and nitric acid in the processes are in accordance with the formula

$$\log \frac{x}{A-x} = K\,(t-t_1)$$

where x is the amount of ammonia and nitric acid which has been produced at time t, A is the total amount of ammonia and nitric acid produced during the process, K is a constant and t_1 is the time at which half of the total amount of ammonia and nitric acid is produced.

Literature Cited

(1) Lemmermann, O., Fischer, H., and Heinitz, B.
1911. Versuche über Stickstoffumsetzung in verschiedenen Böden. *In* Landw. Jahrb., Bd. 41, p. 755-822.

(2) Lipman, J. G., Blair, A. W., Owen, I. L., and McLean, H. C.
1912. Experiments on ammonia formation in the presence of carbohydrates and other non-nitrogenous organic matter. N. J. Agr. Exp. Sta. Bul. 247, 22 p.

(3) Warington, R.
1879. On Nitrification; A Report of Experiments Made in Rothamsted Laboratory. Part 2.

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THE VERTICAL DISTRIBUTION OF PHOSPHORUS IN THE SURFACE SOIL OF PRAIRIES[1]

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INTRODUCTION

The distribution of phosphorus in the successive horizontal layers of the soil is not governed by any universal rule (7, p. 252), the surface soil being sometimes richer and sometimes poorer in this constituent than the corresponding subsoil, as Wohltmann pointed out in connection with a study of the soils of western Germany (9, p. 31). In the Chernozem soils of Russia, according to Kossowitsch (6, p. 327), the amount in the first four to eight inches is somewhat higher than in the underlying layers.

The prairie soils of Illinois (5, p. 82-87) and Iowa (4, p. 138-152) show in most cases a higher percentage in the surface than in either the subsurface or the subsoil, the sections being, respectively, 0 to 6 2/3, 6 2/3 to 20 and 20 to 40 inches.

A recent study of the soils on different parts of the loess in Nebraska (1, p. 302) has shown that in general the proportion in the first and the second foot is smaller than that in the lower layers, and further that when the first is compared with the second foot, now the one and now the other contains the more.

It appears probable that any regularities found in the vertical distribution of phosphorus on the prairie soils of the loess would occur also in any other secondary soil that was originally uniform throughout the surface and lower layers, such as the soil on till plains and lacustral deposits like the clay loams of the Red River Valley, provided that the soil, since its deposition, had borne only a grassland vegetation.

EXPERIMENTAL

Having available a set of inch sections from the surface foot of the same fields on the Nebraska loess which had been sampled for the above-mentioned study, we have determined the total phosphorus by digestion with hydrofluoric and nitric acids (8, p. 163). With most of the samples the datum is the result of a single determination.

The soil samples (2, p. 206) had been collected from 30 virgin prairie fields, five near each of six stations of the United States Weather Bureau—Wauneta, McCook, Holdrege, Hastings, Lincoln and Weeping Water—ranging in annual precipitation from 18.5 to 30.2 inches, and in altitude from 3000 to 1100 feet, the first mentioned station being about

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300 miles west of the last. In each field 20 (and in the fields near Lincoln 50) cores 1 foot long and 1⅜ inches in diameter were taken and subdivided into inch sections. Accordingly, each of the samples prepared for analysis, by combining those from the five fields of an area, was a composite of 100 (or 250) individual samples. In the case of the surface inch section all aerial portions of living plants had been removed but the roots as well as the plant debris upon the surface were treated as integral parts of the soil.

In calcareousness the soils of the different areas show a wide range (3, p. 414). The subsoils at McCook and Wauneta were very calcareous, while those at Lincoln and Weeping Water contained little carbonate. The surface foot in all the fields showed only very small amounts of carbonates, although apparently enough to meet fully the requirements of the most calciphilous of the ordinary farm crops—alfalfa.

It seems safe to assume that the twelve composites from any one of the six areas represent material which at the time of its deposition was uniform in composition, and, accordingly, that any important differences between these now found are to be attributed to subsequent alteration, due to leaching, the action of vegetation, etc.

The data are reported as phosphoric acid in Table I. In all six areas there is a decrease in the phosphorus content from the first to the twelfth inch. In the case of each area there is at least one section that shows a little more or less than corresponds to its position, but as in most cases the datum is the result of a single determination it is not improbable that most, if not all, of these slight irregularities in the steady decrease of phosphorus from the surface downward would have disappeared if duplicate determinations had been made. In the eastern three areas the first section, which carried considerable plant debris, is distinctly richer in phosphorus than the second, while in the western three areas there is little difference. When the distribution is compared by 3-inch, instead of by 1-inch, sections each of the areas shows a regular decrease from the surface downward, and when the upper half of the foot is compared with the lower half, the former shows from 8 to 30 per cent the more.

As in each area the organic matter also decreases steadily throughout the surface foot (2, p. 228) it is of interest to compare the change in the phosphoric acid content with that of the organic matter. In Table II are shown, for the 3-inch sections, the organic matter calculated from the organic carbon (Organic C $\times$ 1.724) as determined by combustion with copper oxide (2, p. 228), and the ratio of organic matter to total phosphoric acid. The ratio decreases from the surface downward and from east to west. Here, as in so many other comparisons (1; 2; 3), the six areas form three groups: the easterly, distinctly humid two, Lincoln and

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Weeping Water, showing a decrease of from 25 to 30 per cent; the westerly, distinctly semi-arid two, McCook and Wauneta, with almost 50 per cent; and the remaining two, occupying an intermediate position. Thus the concentration of phosphorus in the upper layers appears far less marked than that of the organic matter. While the upper layers are the richer in both, the accumulation of the one proceeds independently of the other. In confirmation of this, we may point out that a comparison of the amount of phosphoric acid (1, p. 302) in the surface foot of each of the

TABLE I

PHOSPHORIC ACID IN THE INCH SECTIONS OF THE SURFACE FOOT OF THE DIFFERENT AREAS

Depth Inches	Wauneta	McCook	Holdrege	Hastings	Lincoln	W. Water	Average
	%	%	%	%	%	%	%
1	.150	.140	.178	.150	.168	.178	.161
2	.146	.146	.175	.137	.146	.158	.151
3	.156	.143	.165	.130	.141	.156	.148
4	.143	.146	.165	.131	.130	.150	.144
5	.146	.140	.165	.129	.135	.154	.145
6	.149	.138	.154	.125	.129	.151	.141
7	.133	.137	.131	.118	.127	.146	.132
8	.134	.134	.130	.118	.127	.138	.130
9	.127	.127	.130	.118	.124	.138	.127
10	.134	.131	.133	.108	.119	.129	.126
11	.137	.138	.129	.108	.121	.121	.126
12	.137	.124	.124	.101	.116	.124	.121
Average							
1-3	.151	.143	.173	.139	.152	.164	.153
4-6	.146	.141	.161	.128	.131	.152	.143
7-9	.131	.133	.130	.118	.126	.141	.130
10-12	.136	.131	.129	.106	.119	.125	.124
Average							
1-6	.148	.142	.167	.133	.141	.158	.148
7-12	.133	.132	.129	.112	.122	.133	.127
Average							
1-12	.141	.137	.147	.122	.132	.145	.137

five fields of any one area with that of the organic matter (2, p. 227), shows that the fields richest in the one constituent are liable to be poorest in the other. Accordingly, if in any one of the six areas, we sampled a large number of upland fields and arranged them in the order of their content of organic matter, we should not expect their relative richness in phosphorus to be at all definitely related to this arrangement. However, in the case of each of the fields we should expect to find the successive 3-inch layers of the surface foot to show decreasing amounts of both constituents.

When comparisons are to be made of the amounts of phosphorus in virgin with those in long-cultivated soils, or even in different virgin fields with one another, this marked difference in the phosphorus content of the different layers of the surface foot requires that special attention should be given to the details of sampling. For example, in the case of two fields, one of which has been used only as meadow while the other has for some years been used as a pasture, the latter may have suffered such

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a compacting of the surface layers that an instrument designed to sample to just 6 inches will take from it a distinctly greater amount of soil, with the result that it will show a relatively smaller content of phosporus. In careful studies of changes in composition induced by cultivation, close pasturage, etc., the relative densities of the different samples should be taken into consideration, as has previously been emphasized in connection with nitrogen studies (2, p. 221-225).

TABLE II

RATIO OF ORGANIC MATTER TO PHOSPHORIC ACID IN THE 3-INCH SECTIONS OF THE SURFACE FOOT

ORGANIC MATTER

Depth Inches	Wauneta	McCook	Holdrege	Hastings	Lincoln	W. Water	Average
	%	%	%	%	%	%	%
1-3	3.91	3.60	6.30	5.90	6.70	6.59	5.50
4-6	2.65	2.88	3.81	3.54	4.99	4.91	3.80
7-9	2.19	2.32	2.94	2.90	4.14	4.02	3.08
10-12	1.84	1.85	2.58	2.59	3.42	3.68	2.66
1-6	3.28	3.24	5.05	4.72	5.84	5.75	4.65
7-12	2.01	2.08	2.76	2.74	3.78	3.85	2.87
1-12	2.65	2.66	3.90	3.73	4.81	4.80	3.76
RATIO OF ORGANIC MATTER TO PHOSPHORIC ACID							
1-3	26.0	25.2	36.3	41.9	43.9	40.0	35.5
4-6	18.1	20.4	23.5	27.5	38.0	32.5	26.7
7-9	16.6	17.5	22.5	24.6	32.8	28.6	23.8
10-12	13.5	14.1	20.1	24.4	28.8	29.5	21.7
1-6	22.0	22.8	29.9	34.7	40.9	36.2	31.1
7-12	15.0	15.8	21.3	24.5	30.8	29.0	22.7
1-12	18.5	19.3	25.6	29.6	35.8	32.6	26.9

An explanation of the concentration of the phosphorus in the uppermost layers of the natural grassland soils does not seem difficult to find. The plant roots forage in both surface soil and subsoil, finding the phosphoric acid in the latter as readily available as in the former, or in some cases even more so (1, p. 306-312). The greater portion of that entering the roots will be deposited in the aerial parts of the plants, and later, on the death of the leaves and stems, be left upon the surface. Each succeeding year will witness a new draft upon the phosphorus of the subsoil without any return. The surface nine inches holds the bulk of the roots, with their contained phosphoric acid, partly drawn from the underlying layers, and the nearer the surface the different 3-inch layers are, the more of the plant debris will they receive through surface fissures and the work of insects.

The accumulation of phosphorus in the surface layers of a soil should not be expected to account for all that has been removed from the corresponding subsoil. Water and wind cause a more or less constant ero-

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sion of the superficial phosphorus-rich soil layer and the plant debris and their deposition in valleys or partial removal to the sea.

The influence the wild herbivorous animals may have exerted upon the distribution of the phosphorus merits separate consideration. It is probable that in many species the carcasses with their accumulation of phosphorus were not distributed over upland and valley at all uniformly. In this connection we might allude to the tendency at the present time of the cattle on the ranges of the west to be overtaken by death, in seasons of great drouth, near streams or water-holes, and during unusal winter storms, in sheltered spots.

Summary

Prairie loess soils in the surface foot show a steady decrease in phosphorus from the surface inch downward, independent of the aridity of the climate in which they have formed.

The concentration of phosphorus in the surface layers, while it may be attributed to the prairie vegetation, is not dependent upon a corresponding concentration of organic matter, the difference between the first and the twelfth inch being much the greater with the latter.

In taking samples for comparisons of the phosphorus content of long-cultivated with virgin prairie fields or even of prairie fields with one another it is important to take into consideration the relative densities of the soils.

Literature Cited

(1) Alway, F. J., and Isham, R. M.
1916. The loess soils of the Nebraska portion of the Transition Region: III. Potash, soda and phosphoric acid. *In* Soil Sci., v. 1, no. 4, p. 299-316, 2 fig.

(2) Alway, F. J., and McDole, G. R.
1916. The loess soils of the Nebraska portion of the Transition Region: I. Hygroscopicity, nitrogen and organic carbon. *In* Soil Sci., v. 1, no. 3, p. 197-238, 2 fig., 3 pl.

(3) Alway, F. J., and Rost, C. O.
1916. The loess soils of the Nebraska portion of the Transition Region: IV. Mechanical composition and inorganic constituents. *In* Soil Sci., v. 1, no. 5, p. 405-436, 2 fig.

(4) Brown, P. E.
1914. The fertility in Iowa soils. Iowa Agr. Exp. Sta. Bul. 150, p. 89-152, 1 fig.

(5) Hopkins, C. G.
1910. Soil Fertility and Permanent Agriculture. 653 p. Boston.

(6) Kossowitch, P.
1912. Die Schwarzerde (Tschernoziom). *In* Internat. Mitt. Bodenk., Bd. 1, p. 199-354, 20 fig.

(7) Ramann, E.
1911. Bodenkunde. Ed. 3, 619 p., 61 fig. Berlin.

(8) Washington, H. S.
1910. Manual of the Chemical Analysis of Rocks. Ed. 2, 200 p. New York.

(9) Wohltmann, F.
1901. Das Nährstoff-Kapital west-deutscher Böden, 56 p., 3 fig. Bonn.

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1. **The effect of chemical reagents.**
2. **The behavior of the glaze at high temperatures.**
3. **Constancy of the weight of the vessels.**
4. **Resistance to sudden changes of temperature.**

TEST 1. To Determine the Effect of Chemical Reagents.—The three reagents which are most likely to attack porcelain ware were employed, *i. e.*:—

1. Strong Sulphuric Acid (Sp. Gr. 1.84) at boiling point.
2. 10% Solution of Caustic Soda at 90°-100° C.
3. 10% Solution of Sodium Carbonate at 90°-100° C.

Each of these tests was continued for four hours. Concentration was prevented in the case of Caustic Soda and Sodium Carbonate by conducting the experiment in a closed oven which prevented evaporation. From the loss in weight sustained by the dish and the area wet, the loss per 100 sq. cm. was calculated.

RESULT

With strong Sulphuric Acid loss per 100 sq. cm.—0.00103 gram.
With 10% Caustic Soda Solution loss per 100 sq. cm.—0.0104 gram.
With 10% Sodium Carbonate Solution loss per 100 sq. cm.—0.00103 gram.

TEST 2. To Determine the behavior of the Glaze at High Temperatures.—A dish containing small pieces of broken porcelain from other dishes was subjected to about 1000° C. in a gas muffle for a period of four hours..

RESULT

A slight discoloration took place but the glaze was unaffected and, while pieces of broken dishes adhered to the glaze, they were easily removed by slight pressure of the fingers, leaving the surface unbroken. It will be noted that this test was made very severe and more likely to bring about the destruction of the dish than will be met with in general work.

TEST 3. To Determine the Constancy of the Weight.—For this test crucibles were carefully heated and then weighed. They were then heated for one and one-half hours in a muffle at a temperature of 850° C.

RESULT

Five crucibles gave an average increase in weight of 0.0014 gram. This increase in weight was accompanied by a marked discoloration of the porcelain.

TEST 4. To Determine the Resistance to Sudden Changes of Temperature.—A dish was filled with wax which was melted and heated gradually to a temperature of 160° C. The source of heat was then removed and the dish containing the molten wax was allowed to cool slightly until the temperature of the wax had fallen to 150° C. This procedure was adopted so that the temperature of the dish should equal that of the wax and not higher. When the specified temperature had been attained the dish was plunged into water at 15° C. As it remained unbroken, the test was repeated at temperatures up to 230° C. Dishes were also put in a muffle previously heated to about 850° C. and when hot were taken out with cold iron tongs and placed on a cold surface.

RESULT

In the first test the dishes remained unbroken when heated to a temperature of 230° C. and in the second test were placed on a cold surface without cracking or chipping of the glazed surface.

The above tests, which were conducted in accordance with the specifications of the National Physical Laboratory in some recently published tests for British porcelain ware, do not sufficiently emphasize the property of great resistance to sudden temperature change possessed by the Japanese porcelain as is shown by the following practical tests we recently conducted in a nearby laboratory:

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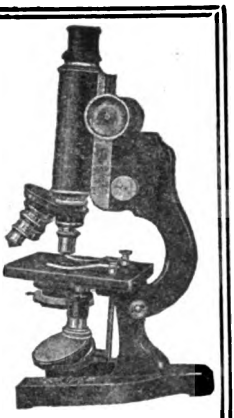

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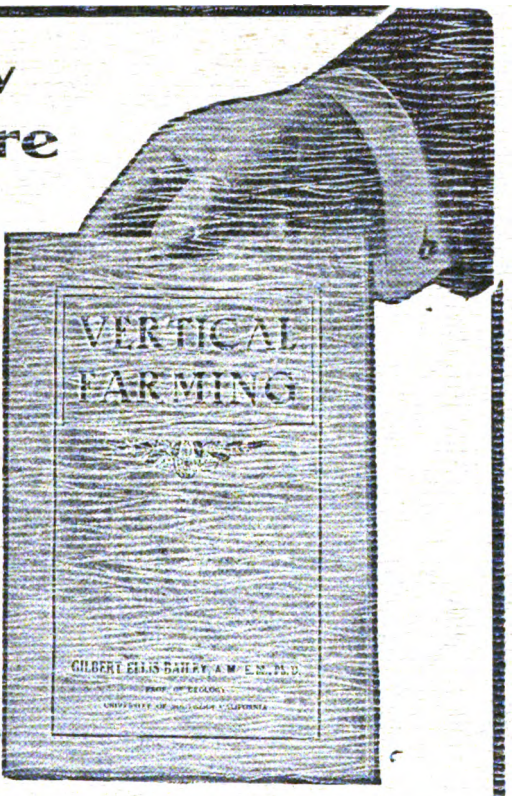

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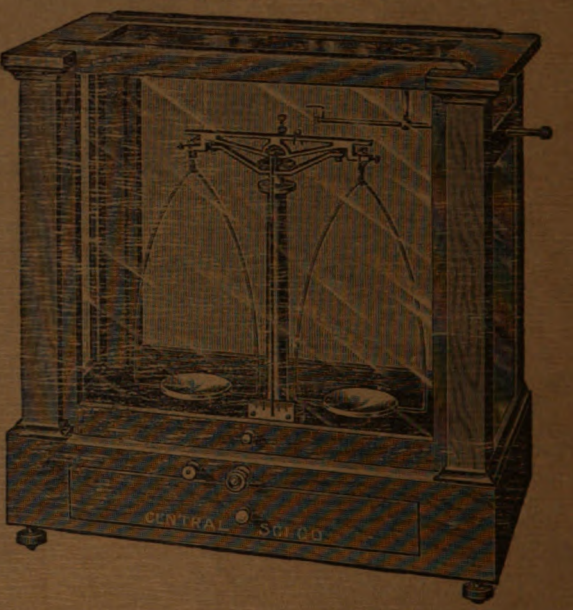

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SULFUR OXIDATION IN SOILS AND ITS EFFECT ON THE AVAILABILITY OF MINERAL PHOSPHATES[1]

BY

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INTRODUCTION

Early in the nineteenth century de Saussure (46) expressed the belief that the ash constituents of plants are indispensable for their growth. Among the ash constituents he detected the constant presence of phosphates and was, therefore, led to assume that phosphorus is an essential ingredient of plant-food. The thought that phosphorus plays a leading role in plant nutrition was further emphasized and developed by Liebig (31). He recognized that a deficiency of this element would necessarily limit production, as is clearly evident from his statement of the law of the minimum. "By the deficiency or absence," he said, "of one necessary constituent, all the others being present, the soil is rendered barren for all those crops to the life of which that one constituent is indispensable."

It was also Liebig's belief that plants could secure their nitrogen from the atmosphere in the form of ammonia, and that a sufficient supply of alkalis, sulfates and phosphates was, therefore, adequate for maximum production under favorable environmental conditions. Moreover, he was led to conclude from the examination of a number of analyses of plant ashes that the mineral requirements differed greatly with the character of the plants themselves and that crops should be supplied liberally with certain mineral ingredients in accordance with their requirements. In its main points Liebig's claim concerning the essential character of phosphorus and of other mineral ingredients in plant nutrition was confirmed by the experiments carried on at Rothamsted under the direction of Lawes and Gilbert. As stated by Russell (44) these experiments had, among other matters, demonstrated by 1855 that "crops require phosphates and salts of alkalis, but the composition of the ash does not afford reliable information as to the amounts of each constituent needed, *e. g.* turnips require

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large amounts of phosphates, although only little is present in their ash. The facts demonstrated by the investigations at Rothamsted are in accord with important evidence contributed from other sources. This evidence includes the numerous sand and water culture experiments performed among others, by Knop, Sachs, Stohmann, Nobbe, Rautenberg, Kühn, Lucanus, W. Wolff, Hampe, Beyer, E. Wolff, Wagner, Bretschneider and Lehmann (1).

In an empirical way the farmers of the Old World recognized the value of certain phosphatic fertilizers long before the beginning of modern research on soils and crops. It is probable that the phosphorus in animal bones and in fish was utilized in crop production in very ancient times. It is also probable that bones were used in England at least occasionally as early as the latter half of the 17th century. The more systematic use of bones was begun about one hundred years later. We are told that: "Col. St. Leger of Wormworth, was the first to introduce bone manuring into England in 1775; but it was many years before their value was fully understood, and it was only in connection with the turnip culture, that they came into general use" (19).

As the use of bones became more common it was recognized that they were slow and uncertain in their action. For instance, it is pointed out by Falkner (15) that: "The smaller the pieces into which the bones are broken, or the nearer to which they are reduced to powder, the more immediate will be their power over the production of the present crop. On clays they are now seldom employed as they show no immediate effect, and require more than light land, but they cannot fail to be ultimately beneficial to the soil." In order to increase the availability of the phosphorus in bone, fermentation in contact with manure and other organic substances was resorted to. In 1829 Anderson of Dundee introduced machinery for grinding bone (15) and thus made their use more effective. A still further step in advance in the utilization of bones as a fertilizer was made when Liebig suggested in 1840 the treatment of bones with sulfuric acid. This was the beginning of the manufacture of dissolved phosphates, or super-phosphates. Soon after that Lawes patented a process for the preparation of super-phosphates out of coprolites and other mineral phosphates. Thus the foundation was laid of a great industry which, in supplying enormous quantities of readily available phosphorus to crops, has made a deep impression on the agriculture of Europe and North America.

Bones served, at first, as the chief source of the super-phosphates sold in England. Later the animal black or animal charcoal from sugar refineries was used as a source of phosphorus. Finally when the possibilities of the large phosphate deposits in the United States were realized, work was started on the manufacture of commercial fertilizers with the mineral phosphates as a source of supply.

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Some interesting information on the early history of the phosphate industry in the United States is furnished by Moses (38). According to him Prof. Shepard and Col. Hatch suggested, in 1859, "the utilization of phosphatic marls in the manufacture of commercial fertilizers, and started a factory at or near Charleston, which was, however, soon abondoned. Remains of their compost heap were utilized by neighboring farmers with good effect long after the war." It appears further, that Dr. St. Julien Ravenel of Charleston engaged in the manufacture of super-phosphates out of European phosphates, soon after the close of the Civil War. When phosphate deposits were discovered in South Carolina in 1867, several companies were organized for their utilization, among them Pratt and Holmes (Charleston Mining Company) and Ravenel and Dukes (Wands Company).

The phosphate deposits of Florida were discovered in 1887 and those of Tennessee in 1894. More recently the United States Geological Survey has prospected and located enormous deposits of phosphate rock in Idaho, Wyoming and Utah. It is estimated that there are in sight 200 million tons in Florida, about 90 million tons in Tennessee and about 30 million tons in South Carolina, Kentucky and Arkansas. These amounts appear insignificant when compared with the five billions of tons of phosphate rock already located in the western states. There is no doubt that other deposits will be located in the Northwest and elsewhere in the United States. But aside from the deposits in North America there are extensive deposits of phosphate rock in Northern Africa, in the Dutch West Indies, on some of the islands in the Southern Pacific, in European Russia and elsewhere.

The Source of Phosphorus in Farm Practices

Where artificial manures are used in European agriculture, acid phosphate, or superphosphate, is supplemented by basic slag, bone meal and phosphatic guano. From the standpoint of quantity only, the basic slag is of large importance. In the United States, on the other hand, basic slag is quite unimportant as a commercial source of phosphorus. By far the greatest proportion of this plant-food constituent is derived here from mineral phosphates after the latter have been acidulated with sulfuric acid. In 1914 there were produced in the United States 1,760,290 tons of superphosphates and 1,116,739 tons of ammoniated superphosphates, aside from the 4,483,465 tons of complete fertilizers. The total fertilizer output of that year was 8,414,959 tons. Among the raw materials employed in the manufacture of these fertilizers were included 2,080,961 tons of phosphate rock, 148,191 tons of bone meal and 1,973,054 tons of sulfuric acid. Since in the manufacture of superphosphate one ton each of sulfuric acid and phosphate rock are used to produce two tons of superphosphate it is evident that there was manufactured in 1914 an equivalent of about 4,000,000 tons of acid phosphate.

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Obviously the cost of acidulation and of the acid itself is one of great economic importance. Acid phosphate usually contains 14 to 15 per cent of phosphoric acid, while the phosphate rock from which it is made contains about 30 per cent phosphoric acid. Hence it is apparent that the so-called available phosphoric acid in acid phosphate is more costly than the inert phosphoric acid in untreated phosphate rock. Furthermore, the greater concentration of the latter would make it relatively cheaper to transport than the acid phosphate. Hence, economy would counsel the use of finely ground phosphate rock, "floats," rather than of acid phosphate, provided that the former could be made to yield rapidly enough an adequate supply of phosphorus to the crop which it is intended to benefit. A discussion of the relative cost and effectiveness of floats and of acidulated phosphate rock would be out of place in this paper. Facts bearing on this question have received consideration among others in the publications of Hall (20), Hopkins (24), Wheeler (56) and Prianishnikov (43). At this time it may be pointed out merely that, while with some soils and crops floats may serve as the most economical and effective source of phosphorus, their use may be desirable only under a fairly limited range of conditions. The limitation of floats may be brought out more clearly in the following statements which seem to be warranted:

1. No natural phosphate is of much value applied directly to the soil except when it is in a state of very fine division.
2. Natural phosphates which consist chiefly of the calcium salt are of very little value when applied directly to soil deficient in organic matter or containing a very large amount of calcium carbonate.
3. Natural calcium phosphate in a finely ground state can be applied with great benefit to soil consisting essentially of vegetable mold or very rich in organic matter.
4. The phosphates of iron and aluminum are less useful than the natural phosphates of calcium.
5. With truck crops, and with most crops grown under intensive conditions raw rock phosphate is unsatisfactory as a phosphorus fertilizer.

In view of the cheapness of tricalcic phosphate as compared with acid phosphate, much thought has been given to the study of methods that could be economically used on the farm for increasing the availability of the former. Taking their cue from the earlier experience with bones, investigators in Europe and America have carried out a large number of composting experiments in which floats were kept for varying lengths of time in contact with decaying organic matter.

Composting Experiments

In 1873 Dietrich (14), who had noticed that applications of raw phosphates produced large increases in crop yield when used on moorland soils started some experiments for the purpose of determining whether the humic acids would have a solvent effect on the phosphates. He made

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up a compost of peat and phosphate rock which was kept moist. Every 14 to 28 days the soluble phosphates were extracted with one liter of water. In a period of 10 to 11 months only 2 to 3 per cent of the phosphoric acid in the rock had become soluble.

A short time later Heiden and Hosaeus (22), working with a low grade phosphate, high in iron, containing 15.6 per cent of Fe_2O_3 and 14.56 per cent of P_2O_5 made three composts which were as follows:

No. 1. 10 parts raw phosphate, 10 parts liquid manure.
No. 2. 10 parts raw phosphate, 20 parts liquid manure.
No. 3. 10 parts raw phosphate, 10 parts stable manure.

Each of these composts was mixed with 36 parts of soil. These mixtures were allowed to compost for about 15 months, and were mixed several times during the period. They were applied as fertilizers on plots. It was concluded that the phosphate was made soluble by composting, but as the check was also manured by mistake, the results obtained were not conclusive. Another experiment was tried in which horse manure was composted with raw phosphate, potassium carbonate and other salts. The results in this case were not definite.

Holdefleiss (23) composted a low grade raw phosphate rock with organic materials such as peat, humus and horse manure. He added to these liquid manure and various salts. The greatest solvent effect was brought about in a compost consisting of 216 kg. of peat, 24 kg. of rock phosphate and 48 liters of liquid manure, and in a compost composed of 216 kg. of peat, 24 kg. of rock phosphate and 5 kg. of ammonium sulfate kept moist with water. The percentages of phosphoric acid made soluble in ammonium citrate were 7.37 and 7.43, respectively.

About eleven years later Fleischer (16) in a study of the action of moorland soils on insoluble phosphate rock, found that the effect was to render a portion of the phosphoric acid soluble in water. Phosphate rock was composted with peat in the proportion of 1 to 5. One experiment was continued for an entire year. The water-soluble phosphoric acid from one compost was 5.51 per cent of the total phosphoric acid added, and the citrate-soluble was 17.08 per cent in one case. Checks containing organic matter only were not included in the experiment so that the results are of very little value.

Kissling (28) continued these investigations. His experiments were designed to study the effect of soluble salts on the solvent action of moorland soils upon phosphate rock. To 20 gm. of air-dried moorland soil there was added rock containing 0.7425 gm. of phosphoric acid. The various salts were added to this mixture. No results of any consequence were obtained. His investigations are open to the same criticism as the work of Fleischer.

Perhaps the earliest experiments conducted in the United States on composting rock phosphate with organic matter were those of Lupton

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(35). In laboratory experiments he found that fermenting cottonseed meal had little solvent effect upon Florida soft phosphate rock or South Carolina ground phosphate rock as determined by the ammonium citrate method. He analyzed portions at intervals for three months. The results obtained were variable.

Pfeiffer and Thurmann (41) found a slight increase in the citrate-soluble phosphoric acid of phosphate rock when composted with peat and liquid manure, and a decided decrease in the citrate-soluble phosphoric acid of superphosphate showing that the formation of tricalcium phosphate or of organic phosphorus compounds must have taken place.

At the Ohio Experiment Station (40) a series of tests has been carried out on the value of floats and of other phosphates for reinforcing manure. In these tests floats were used at the rate of 40 pounds per ton of barnyard manure. The latter was allowed to compost for a few weeks, and was then used in plot experiments. A very slight increase in crop yield was obtained over the plots receiving uncomposted manure and floats.

Also Shutt (48) found fermenting manures to have only a slight solvent action on composted rock phosphate (apatite).

McDowell (36) composted six pounds of Tennessee rock phosphate with 228 pounds of stable manure. The floats were applied in alternate layers. After fermenting for about 13 months analyses showed no increase in either the water or citrate-soluble phosphoric acid, a condition which confirmed the results of Lupton.

Truog (53) in laboratory experiments in which floats, manure and grass were composted together, found that there was little or no solvent action of the fermenting material. In pot experiments, when the floats were thoroughly mixed with soil, favorable results were obtained, indicating that carbon dioxide given off by plant roots was instrumental in breaking down the insoluble phosphate. Where manure was added carbon dioxide production was increased; this was accompanied by a greater solvent action on the floats. He concludes that in composting experiments the dissolved substances are not removed as under field conditions; the laboratory experiments therefore fail to imitate field conditions.

Hartwell and Pember (21), in order to determine whether cow dung had any solvent action on phosphate rock, made up composts containing the two materials and allowed them to ferment for several months. The availability of the phosphate in the composts was determined by comparing the yields of millet and oats grown in duplicate pots. In summing up the results obtained they concluded, "The experiments indicate that practically no increase in the amount of available phosphorus results from mixing fresh cow dung and floats together for a number of months before applying them to the land."

The more recent experiments of Tottingham and Hoffmann (52) show that after a fermentation of 4 to 6 months the water-soluble phosphoric acid in manure and manure-floats mixture decreased. Where bac-

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teria were active a saturated solution of carbon dioxide had no better action than water for extracting phosphoric acid from the mixture; but at the end of 10 months when the bacteria in the composts were practically inert it gave superior results. Acid phosphate underwent the same changes in solubility as the rock phosphate.

Other Experiments Relating to the Availability of Insoluble Phosphates

The solvent effect of different so-called "physiologically acid" salts on raw phosphate has been the subject of much experimental inquiry.

In 1900, Warington (55) called attention to the fact that certain lime phosphates were made more available to plants when used together with ammonium sulfate.

Experiments carried out by Schulov (47) in 1900 and later show that ammonium sulfate and ammonium nitrate furthered the assimilation of phosphoric acid by plants from finely ground phosphorite. He ascribed this action as being due to the so-called "physiologically acid" nature of the ammonium salts. On the other hand experiments carried out with calcium nitrate showed little solvent effect on the phosphorites. He designated this salt as being "physiologically alkaline." The plants apparently assimilated the basic radicle and left the acid radicle in the soil, which would have a slight solvent effect on the phosphorite.

Other work along this line has been carried out by Kossovich (30), Prianishnikov (42), Söderbaum (50), Nedokuchaev (39), Mitscherlich and Simmermacher (37).

We may also note here Hopkins' (24) suggestion that some of the favorable results obtained with floats may be partially due to the formation of nitric acid in the course of the nitrification processes going on in the soil, which would have a soluble effect on the insoluble phosphates present.

The investigations concerning the action of decay bacteria and of other microörganisms on the mineral phosphates of the soil have not been very extensive. Work in this field has been stimulated by the knowledge that carbon dioxide is formed in large quantities in all processes of decay and putrefaction, and that the carbon dioxide thus formed when dissolved in water should hasten the solution of inert phosphates. Liebig himself pointed out (32) the probable action of carbon dioxide generated in the soil, on the solution of phosphates. Experimentally, this question was taken up by Bretschneider (6) in 1870. Indications of some solvent effect were found when phosphates were acted upon by water, and by water saturated with carbon dioxide. Similar tests were made by Karmrodt (27). The materials used by him included phosphate rock, bone ash and raw bone meal. When these materials were kept in contact with water saturated with carbon dioxide for a period of about 6 weeks a marked solvent effect was noted. In the case of the phosphate rock about 9½ per cent of the phosphoric acid had become soluble.

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In 1900 Stoklasa (51) suggested that the solution of phosphoric acid in bone meal may be increased by bacteria.

Koch and Kröber (29) found that a number of insoluble phosphates were rendered soluble by the acids formed by sewage and soil bacteria from dextrose.

Sackett, Patten and Brown (45) and Brown (8) have found that certain soil bacteria exert a solvent action on raw phosphate rock, and other insoluble phosphates. They concluded that the carbon dioxide liberated in the medium by bacterial activities was the important factor, although acids formed by certain bacteria produced a slight solvent effect.

Recent investigations by Bassalik (3) show that certain bacteria have a solvent effect on apatite and other silicates. Nitrite bacteria showed an appreciable solvent action notwithstanding the slight production of acid. Butyric acid bacteria also exercised a solvent effect on apatite.

More recent investigations carried out by Hopkins and Whiting (25) corroborate the results obtained by Bassalik, in that the nitrous acid formed by nitrite bacteria was found to exert a solvent effect on pulverized rock phosphate.

Some work has also been done on the effect of iron sulfide and ferrous sulfate on insoluble phosphates. Mention may be made in this connection of the studies of Cazeneuve and Nicolle (10) who made tests on the solvent effect of ferrous sulfate on various phosphatic fertilizers, including mineral phosphate. After 8 days the mineral phosphate had suffered no change in solubility as measured by acetic acid and ammonium citrate.

Boiret and Paturel (4), using ferrous sulfate in water culture tests on plants, found that sulfuric acid was formed and the plants were consequently killed. Tests made in soils showed that the ferrous sulfate exerted no effect on the amount of phosphoric acid in the product. The ferrous sulfate in this case was not used in conjunction with other fertilizers.

Recent investigations by Galtsev and Yakushkin (18) on the combined action of phosphorite, and of iron sulfide and its oxidation products show that modified pyrite containing sulfate and sulfuric acid, produced beneficial effects on crop yields, when used in pot experiments with phosphorite, indicating a partial solution of the insoluble phosphate.

The Oxidation of Sulfur by Microörganisms in its Relation to the Availability of Phosphates

A series of experiments by European and American investigators on the oxidation of sulfur by microörganisms suggested to the senior author of this paper that this reaction might be of possible value in making available the phosphorus of mineral phosphates. Accordingly, experiments were planned and started in the fall of 1915. A preliminary report on these experiments was made in a recent issue of SOIL SCIENCE (34). In

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the present paper the data thus far obtained are discussed at greater length. But prior to their consideration in the following pages it seems desirable for the sake of completeness to refer to certain publications which have a more or less direct bearing on the problem.

As early as 1877 it was suggested by Charles F. Panknin of Charleston, South Carolina, that sulfur when mixed with ground bone or ground mineral phosphates would be oxidized to sulfuric acid when incorporated into cultivated soil. He applied for a patent on his process in April, 1877, and succeeded in August of the same year in securing Letters Patent 193,890. In his application for the patent Panknin made the following statement: "My invention relates to a compound consisting of a mixture of finely-powdered bone or mineral phosphates and finely-powdered sulphur, the said ingredients being mixed together and applied in a dry state, and the conversion of the insoluble phosphate of lime is affected by the sulphuric acid eliminated by the natural oxidation of the sulphur of the mixture in the soil to which it is applied."

"To prepare the fertilizing compound, take 95 parts of the insoluble phosphates, (either bone or mineral phosphate,) in a finely powdered state, and mix with it 5 parts of finely-powdered sulphur, both ingredients being in a dry state, though I do not confine myself to these proportions. This mixture though only mechanical in its character, when applied to the soil, becomes slowly transformed, the sulphur being first converted into sulphuric acid by a natural process of oxidation, and when so converted, uniting with the insoluble phosphates to form a soluble phosphate, which is dissolved by the moisture of the soil, and acts in the same manner as phosphates made by artificial process."

In September, 1904, a patent application was submitted by William B. Chisholm of Charlestown, South Carolina, and Patent 824,280 was secured by him on June 26, 1906. Chisholm's invention as described by him "relates to certain new and useful improvements in fertilizers and has for its object the production of a fertilizer which in part subserves the functions of a germicide and which, while of greatly decreased cost of production in comparison with the ordinary phosphatic fertilizers now on the market, is of largely increased efficiency in use and is practically without destructive effect upon any kind of crop or of the bagging or the like in which it may be stored or transported." It is further stated by Chisholm that in the preparing of his fertilizer "the phosphate rock and the sulfur are first passed together through a preliminary crusher, reducing them to the average size of, say, a pea or bean. The crushed rough mixture is then admitted into a pulverizing mill of the Lucop or other suitable type and is reduced there in to an impalpable powder, any tailings being returned to the mill for further grinding. The resultant is an intimate and uniform admixture of phosphate rock and sulfur in the form of an almost impalpable powder and in an absolutely dry condition. I find that the percentage of sulfur, by weight, to give effective results may in some

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instances be as low as forty pounds to the ton of the mixture—*i. e.*, forty pounds of sulfur to nineteen hundred and sixty pounds of phosphate rock—and in other instances from sixty to one hundred pounds of sulfur to the ton of mixture. . . . In the practical use of my invention I find that the crops produced are apparently far superior to what might reasonably be expected from the amount of available phosphate, nitrogen, and potash or other ingredients present in the final mixture. This may be due in part to the extreme fineness and intimate association of the admixed particles tending to facilitate their ready and uniform distribution through the soil, their chemical interaction under the influence of the moisture of the soil and the infiltrations of rain, and their increased solubility in said condition of impalpable powder; but I am further led to believe that the sulfur itself has an independent effect as a germicide, destroying bacterial and insect life prejudicial to the plant and either not interferring with or positively stimulating helpful bacteria."

The above citations from the Panknin and Chisolm patents bring out some very interesting facts. It is evident that Panknin knew nearly forty years ago that sulfur when mixed with soil may be changed into sulfuric acid. Moreover he recognized the value of sulfur oxidation for transforming insoluble into soluble phosphates. He did not know, of course, that the process of sulfur oxidation is largely biological in character. Neither did he recognize that the very small quantities of sulfur recommended by him, namely one part to twenty parts of phosphate were entirely inadequate for accomplishing the desired reaction. The same criticism may be made of the process proposed by Chisolm. In both instances relatively small amounts of sulfur are directly applied to the soil with the expectation that the sulfuric acid ultimately produced would attack the insoluble phosphates. The fact was obviously overlooked by both Panknin and Chisolm that when twenty to one hundred pounds of sulfur are mixed with two to three million pounds of soil the basic substances in the latter including compounds of lime, magnesia, iron and alumina would readily react with the sulfuric acid derived from the oxidation of sulfur and no free acid would be left for any appreciable action on the insoluble phosphates. This fact was duly appreciated by the authors of this paper when the experiments described in the following pages were being planned, even though they did not know at that time, of the existence of the Panknin and Chisolm patents. It seemed to them that the inert phosphates could be best made available by means of sulfur oxidation in a relatively concentrated medium *e. g.* in a compost heap containing a relatively large proportion of both phosphate and sulfur. The data recorded below show that their reasoning was sound, as will be shown at greater length elsewhere in this paper. Meanwhile it would not be out of place to mention at least briefly the recent studies on the oxidation of sulfur by microörganisms.

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In attempting to determine the value of sulfur as a fertilizer, Degrully (11) discovered in 1911, that the sulfur applied at the rate of 109 gm. per square meter was largely oxidized to sulfates. Somewhat later Boullanger (5) was able to prove a favorable effect of sulfur on crop yields. Since the corresponding results in sterilized soil were not marked, Boullanger concluded that the oxidation of sulfur is brought about by biological activities. The results just noted were confirmed by Demolon (12) in 1912 and again in 1913 (13).

In his earlier experiments he employed 5 gm. of sulfur to each 100-gm. portion of soil. Notwithstanding these relatively large applications, much of the sulfur was oxidized to sulfuric acid. Subsequently, Demolon added 1 per cent of sulfur to garden soil and kept the mixture moist and at a temperature of 20° C. Under these conditions appreciable quantities of sulfuric acid were formed, as shown by the amounts of calcium sulfate found in the mixture at the end of 40 days. It was observed at the same time that the oxidation of sulfur was most active in a non-sterilized soil. In fertilizer experiments it was found that when sulfur was supplied in an amount equal to that of the nitrogen, the yields were as good as those obtained from a complete fertilizer. He ascribed the beneficial action of sulfur as having been due to its influence on the activities of soil bacteria, as well as to its gradual transformation into sulfuric acid and the reaction of the latter with basic material to form an increased supply of mineral plant-food.

The oxidation of sulfur was made the subject of study also by Brioux and Guerbet (7). They dealt particularly with the influence exerted on the oxidation of sulfur by the soil itself and by certain carbohydrates, peptone and other nitrogenous materials. Their investigations showed that carbohydrates had a retarding effect, while peptone and certain other nitrogenous materials accelerated it so that in 30 days 82 per cent was found to have been oxidized to sulfates. Calcium carbonate was also found to have enhanced the rate of sulfur-oxidation. They concluded, therefore, that the oxidation of sulfur in soils is a very complicated process and involves the activities of a number of species of bacteria.

Vermorel and Dantony (54), in studying the fertilizing action of sulfur and iron sulfide on wheat and beans obtained increased yields when organic matter was present. The experiment indicates that the activities of sulfofying bacteria are stimulated by organic matter.

Some interesting data concerning the influence of sulfur on soil acidity are recorded by Lint (33). In order to ascertain the rate of oxidation of sulfur in soil, 100-gm. portions of it were treated with 33 mg. of flowers of sulfur, which is an addition equivalent to 100 pounds of sulfur per acre foot of soil. The moisture content was 20 per cent. The acidity was found to increase up to the eighth week when all the sulfur was apparently oxidized. Experiments with a heavy clay loam and a sandy loam soil showed a much more rapid oxidation of the sulfur in the latter

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soil. Soils with a water content of 20 per cent when allowed to dry did not cause as rapid an oxidation as those maintained at an optimum moisture content throughout the experiment.

Shedd (49) studied the oxidation of sulfur in soil and sand. With soil to which sulfur was added in amounts varying from 100 pounds to 7000 pounds per acre, he found by analysis, that 60 to 80 per cent of the sulfur, regardless of the amount added was oxidized after a period of about 4 months. Sulfur was oxidized also in the sand but not to the extent that it was in the soil.

Kappen and Quensell (26) made a study of the sulfur cycle in soils. In connection with these experiments they studied the oxidation of ground rhombic sulfur and the so-called "Schwefel milch." The latter was prepared by mixing together 2 parts of sulfur, 1 part of calcium hydroxide and 13 parts of water. The solution was heated until the constituents dissolved. After diluting and precipitating with dilute hydrochloric acid, the precipitated sulfur was filtered and washed. Two hundred mg. of the dry sulfur thus prepared and of the ground rhombic sulfur were mixed with the different soils under investigation, incubated for six weeks, extracted with 10 per cent of hydrochloric acid and the sulfates determined in the extract. The results which they obtained are quoted below to show the striking oxidation of the precipitated sulfur.

Soil	SO_4 from 200 mg. of Sulfur	
	Rhombic Sulfur	Precipitated Sulfur
Glass Sand	3.69 mg.	44.63 mg.
Sandy Soil	3.81 mg.	144.22 mg.
Loam Soil	16.44 mg.	227.57 mg.
Meadow Soil	10.91 mg.	284.01 mg.

Brown and Kellogg (9) report some experiments on the so-called sulfofying power of soils. They find that each soil has a definite sulfofying power. Organic matter was found to increase the amount of sulfur oxidized. Manure and green manure increased it up to a certain point. Moisture was found to play an important part in the oxidation process, and the optimum moisture condition was apparently 50 per cent of complete saturation. Other experiments which they carried out show that aeration is an important factor, as is also the absence of carbohydrates. The presence of bacteria is essential, although the production of a small amount sulfates was brought about by chemical action.

Ames and Boltz (2) in their recent study of the effect of sulfur on the growth of plants, found that it produced increased yields when applied to certain crops. In order to determine whether or not this beneficial action was due to the solvent action on the mineral soil constituents of the acids produced during the sulfofying processes, they conducted a composting experiment in which sterilized and unsterilized soils, a loam and a clay soil, and pure quartz sand were used. To 500-gm. portions of the soil and sand were added 0.25 gm. of flowers of sulfur and Tennessee phosphate

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rock in amounts containing 0.06 gm. of phosphorus. To each mixture was added 400 c.c. of distilled water. After 2 months they found but a slight increase in the availability of the phosphorous, as measured by 0.2 per cent hydrochloric acid. The acidity of the mixture had also increased but little.

These investigators have evidently assumed that sulfofication is a process brought about under anaerobic conditions; while the experiments of Brown and Kellogg (9) and those of Lipman, McLean and Lint (34) show that aerobic conditions favor the most rapid oxidation of sulfur in the soil.

EXPERIMENTAL

Methods

Composts. In making up the composts 100-gm. portions of soil were placed in tumblers, the phosphate rock and sulfur added together with other materials, if such were used in the experiment, and the whole thoroughly mixed. In all cases the composts were arranged in duplicate and duplicate analyses made. Moisture was then added and the tumblers were covered with petri dishes and placed in a dark closet kept at a temperature of 22° to 24° C. The different composts were weighed and water was added up to the optimum every two weeks.

Sampling. In order to obviate the need of making moisture determinations for each analysis, the entire amount of soil from each tumbler was placed upon the scale pan, weighed and one-fifth of the total weigth taken for each determination.

Ammonium-Citrate-Soluble Phosphoric Acid. The sample of compost was transferred to a 500 c.c. volumetric flask with 100 c.c. of neutral ammonium citrate solution which had been heated to 65° C. The extraction was made for 30 minutes in a water bath kept at a temperature of 65° C. The flasks were cooled and made up to volume with distilled water. After thorough shaking, the extract was filtered and an aliquot representing 1/50 of the entire compost taken for analysis. This aliquot was transferred to a 400-c.c. beaker and evaporated to dryness with aqua regia several times, in order to oxidize the organic matter, and to render insoluble any silica which might be in solution. The residue was taken up with acid and the phosphoric acid determined in the usual manner by the volumetric method.

Water-Soluble Phosphoric Acid. The sample of compost taken for analysis was transferred to a filter with hot water, and thoroughly washed. The washing was repeated until the filtrate measured 500 c.c. After cooling, making up to volume and vigorously shaking, an aliquot representing 2/25 of the total compost was transferred to a 250-c.c. beaker, a few drops of nitric acid were added, the contents of the beaker were evaporated to a volume of 25 c.c., and the phosphoric acid determined in the usual manner.

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Acidity (Relative). For determining the relative acidity the following method was used. One-fifth of the compost from each tumbler was transferred to a 200-c.c. volumetric flask with 160 c.c of hot distilled water, thoroughly shaken every five minutes throughout a period of one-half hour, after which the contents of the flasks were cooled, made up to volume and thoroughly shaken. An aliquot was drawn off, boiled for 5 minutes to drive off any carbon dioxide which might be present, phenolphthalein was added and the liquid titrated to a faint pink color with N/50 KOH.

Sulfates. Five-gm. portions of the composts were transferred to flasks, with 100 c.c. of 1 per cent hydrochloric acid, placed on a shaking machine and shaken for 7 hours. The sulfates were then determined in aliquots by the usual method.

Oxidation of Sulfur in Different Soils as Affecting the Availability of Rock Phosphate

Since different soils have been found by Brown and Kellogg (9) and by Kappen and Quensell (26) to show differences in their ability to oxidize sulfur, this experiment provides for the use of two soils and of sea sand. One of these, a greenhouse soil, was a red silt loam soil to which had been added composted manure. It contained .2970 per cent of P_2O_5, 10.14 per cent of Fe_2O_3 and Al_2O_3, and 3.04 per cent of organic carbon. The other, a Sassafras loam soil contained 1.07 per cent of organic carbon, 5.39 per cent of Fe_2O_3 and Al_2O_3 and 0.1950 per cent of P_2O_5. The soils were air dried and, for the sake of uniformity, passed through a 10-mesh sieve. The phosphate rock, or floats, used in this experiment was Tennessee brown rock phosphate containing 30 per cent of total P_2O_5 and ground so that 95 per cent would pass through a sieve with 10,000 meshes to the square inch. The 15-gm. portion of the rock phosphate used in each compost contained 4500 mg. of P_2O_5. Two forms of sulfur were employed: flowers of sulfur and precipitated sulfur; the latter was prepared according to the method of Kappen and Quensell (26).

The composts of soil, rock phosphate and sulfur were made according to the plan given, transferred to tumblers, thoroughly mixed, and water added in amounts equivalent to 50 per cent of the water-holding capacity. In order to insure proper inoculation and to supply mineral food to the bacteria in the sand mixtures, 10 c.c. of the water were added in the form of soil infusion, obtained by shaking for 10 minutes 100 gm. fertile soil with 200 c.c. of a dilute synthetic culture medium containing no phosphorus. All the tumblers were then weighed and the weights recorded on each, so that any water lost by evaporation could be replaced. They were then covered with petri dishes and placed in a dark closet, which was kept at a temperature of 22° to 24° C.

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For the study of the weekly changes in the various composts, analyses were made at the beginning of the experiment and at the end of each week, up to the eighth week; after that the analyses were made at the end of every two weeks up to the twelfth week, after which the composts were allowed to stand for a longer period. The water-soluble phosphoric acid in the mixtures was determined at different intervals. The weekly changes in the citrate-soluble phosphoric acid and acidity of the composts are recorded in Tables I, II and III. In figure 1 the results are shown graphically.

Plan of Experiment

Several sets of this experiment were arranged in duplicate according to the following plan.

TREATMENT

Lab. No. (Optimum moisture added to each)

1. 100 gm. Sea Sand, inoculated with soil infusion.
2. Same as No. 1., 5 gm. Flowers of Sulfur added.
3. Same as No. 1., 15 gm. Floats added.
4. Same as No. 3., 5 gm. Flowers of Sulfur added.
5. 100 gm. Greenhouse Soil.
6. Same as No. 5, 5 gm. Flowers of Sulfur added.
7. Same as No. 5, 15 gm. Floats added.
8. Same as No. 7, 5 gm. Flowers of Sulfur added.
9. 100 gm. Sandy Loam Soil.
10. Same as No. 9, 5 gm. Flowers of Sulfur added.
11. Same as No. 9, 15 gm. Floats added.
12. Same as No. 11, 5 gm. Flowers of Sulfur added.
13. Same as No. 9, 5 gm. Precipitated Sulfur added.
14. Same as No. 13, 15 gm. Floats added.

The data included in Tables I, II and III show that as a result of the oxidation of the sulfur there was not only an increase in the acidity of certain of the mixtures but also an accumulation of water-soluble and citrate-soluble phosphoric acid when the sulfur and tricalcic phosphate were used together.

A careful comparison of the results recorded in these tables will show some very definite differences as brought about by treatment, as well as by variation in the physical and chemical properties of the soils used. It will be noted, among other matters, that in the sand mixtures there was practically no change in the amounts of acid and of available phosphorus when neither sulfur nor tricalcic phosphate was employed. The initial acidity was here equivalent to 6 c.c. of N/50 KOH. At the end of the third week there was a slight increase to an equivalent to 8 c.c. After that there were important fluctuations to the end of the thirtieth week when the amount found was equivalent to 7 c.c. of N/50 KOH. Similarly,

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but slight changes occurred in these mixtures in the content of available phosphoric acid. The greatest variation was found at the end of the seventh week, when 25.39 mg. of citrate-soluble phosphoric acid were present, as against 34.58 mg. at the beginning of the experiment. The decrease in this instance was probably due to the assimilation of a portion of the available phosphorus by soil microörganisms.

The sand mixtures which received additions of sulfur alone showed a progressive increase in acidity until the end of the eighteenth week. Lack of material prevented the making of corresponding determinations at the end of the twenty-fourth and thirtieth weeks. It will be observed,

TABLE I

SAND: CHANGES IN ACIDITY AND AVAILABLE PHOSPHORIC ACID

Time	Additions							
	None		5 gm. Flowers of Sulfur		15 gm. Floats		5 gm. Fl. of Sulfur 15 gm. Floats	
	Acidity c.c. N/50 KOH	P_2O_5 mg.	Acidity c.c. N/50 KOH	P_2O_5 mg.	Acidity c.c. N/50 KOH	P_2O_5 mg.	Acidity c.c. N/50 KOH	P_2O_5 mg.
Beginning	6.00	34.58	7.25	35.06	5.75	136.62	7.50	139.04
End of 1st week....	6.00	33.85	12.00	35.06	6.00	169.26	9.50	171.68
End of 2nd week....	.....	.....	.....	.....	7.25	139.00	21.50	180.38
End of 3rd week....	8.00	32.64	34.50	33.37	7.50	177.72	46.50	160.80
End of 4th week....	.....	.....	.....	.....	8.25	187.39	143.00	234.55
End of 5th week....	6.50	27.81	41.50	28.23	6.50	168.05	174.50	224.82
End of 6th week....	.....	.....	.....	.....	7.75	122.10	177.00	272.02
End of 7th week....	7.50	25.39	58.00	24.18	9.50	136.62	215.00	258.73
End of 8th week....	.....	.....	.....	.....	7.00	136.70	208.00	262.87
End of 10th week....	7.50	31.43	96.00	34.52	7.50	136.62	338.00	337.31
End of 12th week....	.....	.....	96.00	.....	7.00	138.20	336.00	390.62
End of 15th week....	.....	.....	100.00	33.80	6.85	116.47	328.50	400.68
End of 18th week....	7.20	27.90	100.00	28.90	7.10	140.20	414.00	455.95
End of 24th week....	.....	.....	.....	.....	7.30	127.80	932.00	949.07
End of 30th week....	7.00	30.82	.....	.....	7.10	129.30	976.00	1115.10
Increase	.....	.....	.....	.....	.....	.....	968.50	966.06

nevertheless, that there was here an increase from an initial acidity equivalent to 7.25 c.c. of N/50 KOH to an equivalent 100 c.c. of N/50 KOH. It is safe to assume, therefore, that notwithstanding the poverty of the culture medium in nitrogen and phosphorus compounds the sulfofying organisms multiplied sufficiently to convert an appreciable amount of sulfur into sulfuric acid. On the other hand, the citrate-soluble phosphoric acid remained practically constant in amount. Obviously the small quantities of sulfuric acid that had accumulated in the sand portions found no phosphates to act on. Thus at the end of the eighteenth week the sulfur-sand mixtures contained an equivalent of 28.90 c.c. of N/50 KOH, as against 27.90 c.c. N/50 KOH, in the sand alone.

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In the floats-sand mixtures there were insignificant changes in the acidity of the medium from the first to the thirtieth week. Moreover, there was a limited increase followed by a decrease in the amount of citrate-soluble phosphoric acid. The initial content of citrate-soluble phosphoric acid was, in these mixtures, 136.62 mg. At the end of the fourth week it was 187.39 mg. and at the end of the thirtieth week 129.30 mg. In this case the absence of acid prevented the accumulation of soluble phosphates beyond the amount originally present in the 15 gm. of floats.

In the sulfur-floats-sand mixtures the conditions were different from those in the sand, the sulfur-sand or the floats-sand mixtures. Here the presence of tricalcic phosphate favored the development of the sulfofy-

TABLE II

GREENHOUSE SOIL: CHANGES IN ACIDITY AND AVAILABLE PHOSPHORIC ACID

Time	Additions							
	None		5 gm. Flowers of Sulfur		15 gm. Floats		5 gm. Fl. of Sulfur 15 gm. Floats	
	Acidity c.c. N/50 KOH	P_2O_5 mg.	Acidity c.c. N/50 KOH	P_2O_5 mg.	Acidity c.c. N/50 KOH	P_2O_5 mg.	Acidity c.c. N/50 KOH	P_2O_5 mg.
Beginning	2.50	111.95	5.00	105.18	4.25	143.39	5.50	142.66
End of 1st week....	2.00	111.71	8.75	96.72	4.25	166.84	11.50	166.84
End of 2nd week....	.....	.....	.....	.....	3.00	176.51	18.75	203.11
End of 3rd week....	4.75	122.11	42.75	120.90	6.25	191.02	89.00	272.75
End of 4th week....	.....	.....	.....	.....	6.00	206.74	224.00	227.29
End of 5th week....	2.75	79.79	137.00	100.34	2.25	166.13	209.00	230.92
End of 6th week....	.....	.....	.....	.....	6.00	148.21	281.00	258.73
End of 7th week....	5.25	83.42	310.60	103.49	6.00	157.17	324.00	253.89
End of 8th week....	.....	.....	.....	.....	5.50	160.79	352.00	320.38
End of 10th week....	6.50	105.18	4404.00	154.75	5.50	153.54	646.00	340.94
End of 12th week....	.....	.....	4560.00	.....	.....	.....	596.00	396.88
End of 15th week....	.....	.....	4700.00	160.10	4.96	138.77	710.00	1982.40
End of 18th week....	3.98	100.00	4690.00	150.20	6.00	149.62	2348.00	2605.62
End of 24th week....	.....	.....	.....	.....	.....	.....	2402.00	3840.90
End of 30th week....	6.00	87.90	.....	.....	5.13	160.40	2400.00	4126.48
Increase	.....	.....	.....	.....	.....	.....	2394.50	3983.82

ing organisms by supplying the element phosphorus essential for their development, and, perhaps, also by preventing the accumulation of too great an amount of free acid in the moisture films surrounding the sand grains. To avoid misunderstanding it should be added here that the amounts of acid as shown by titration of the sulfur-floats-sand mixtures were probably due to acid salts rather than free sulfuric acid.

It is worth while to note here that the increase in the acidity equivalents was striking and suggestive. At the end of the third week the acidity found was equivalent to 46.50 c.c. of N/50 KOH. In the

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TABLE III

SASSAFRAS LOAM SOIL: CHANGES IN ACIDITY AND AVAILABLE PHOSPHORIC ACID

Time	Additions											
	None		5 gm. Flowers of Sulfur		15 gm Floats		5 gm. Flowers of Sulfur 15 gm. Floats		5 gm. Precipitated Sulfur,		5 gm. Precipitated Sulfur, 15 gm. Floats	
	Acidity c.c N/50 KOH	P_2O_5 mg.	Acidity c.c N/50 KOH	P_2O_5 mg.	Acidity c.c N/50 KOH	P_2O_5 mg.	Acidity c.c N/50 KOH	P_2O_5 mg.	Acidity c.c N/50 KOH	P_2O_5 mg.	Acidity c.c N/50 KOH	P_2O_5 mg.
Beginning	6.00	79.79	6.50	83.42	5.55	155.96	7.50	154.27	8.50	83.75	8.75	143.87
End of 1st week	9.50	77.37	11.25	94.30	10.00	162.01	10.00	164.91	12.25	96.73	14.30	169.26
End of 2nd week	.....	.....	.....	.....	10.00	191.01	11.50	222.46	.....	.....	16.25	186.19
End of 3rd week	6.00	120.90	33.50	125.74	6.25	171.68	107.50	209.16	30.25	132.99	130.25	247.85
End of 4th week	.....	.....	.....	.....	7.00	180.14	360.50	269.61	.....	.....	378.50	264.77
End of 5th week	7.50	99.14	450.50	103.97	5.25	165.63	353.00	342.15	387.00	94.30	414.00	413.48
End of 6th week	.....	.....	.....	.....	6.50	155.75	421.00	406.22	.....	.....	417.50	425.57
End of 7th week	7.00	71.33	597.00	89.47	10.50	158.38	485.00	339.73	735.00	95.51	465.00	303.46
End of 8th week	.....	.....	.....	.....	8.50	175.67	478.00	508.98	.....	.....	475.00	510.92
End of 10th week	7.00	96.72	1240.00	100.35	8.50	158.86	660.00	518.66	1216.00	91.89	640.00	535.59
End of 12th week	.....	.....	1300.00	.....	.....	.....	637.00	655.20	1228.00	.....	604.00	597.20
End of 15th week	.....	.....	1440.00	101.40	7.45	168.50	570.25	867.30	.....	96.10	600.25	670.30
End of 18th week	6.95	80.20	1590.00	100.00	7.50	161.30	530.00	612.07	1858.00	98.70	756.00	966.42
End of 24th week	.....	.....	.....	.....	.....	.....	534.00	688.88	.....	.....	576.00	662.87
End of 30th week	7.10	75.15	.....	.....	6.70	170.81	690.00	822.70	.....	.....	726.00	783.05
Increase	.....	.....	.....	.....	.....	.....	682.50	668.43	.....	.....	717.25	639.18

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sulfur-sand mixture the corresponding equivalent was only 34.50 c.c. N/50 KOH. At the end of the fifth week the acidity equivalents in the sulfur-sand and sulfur-floats-sand mixtures were 41.50 c.c. and

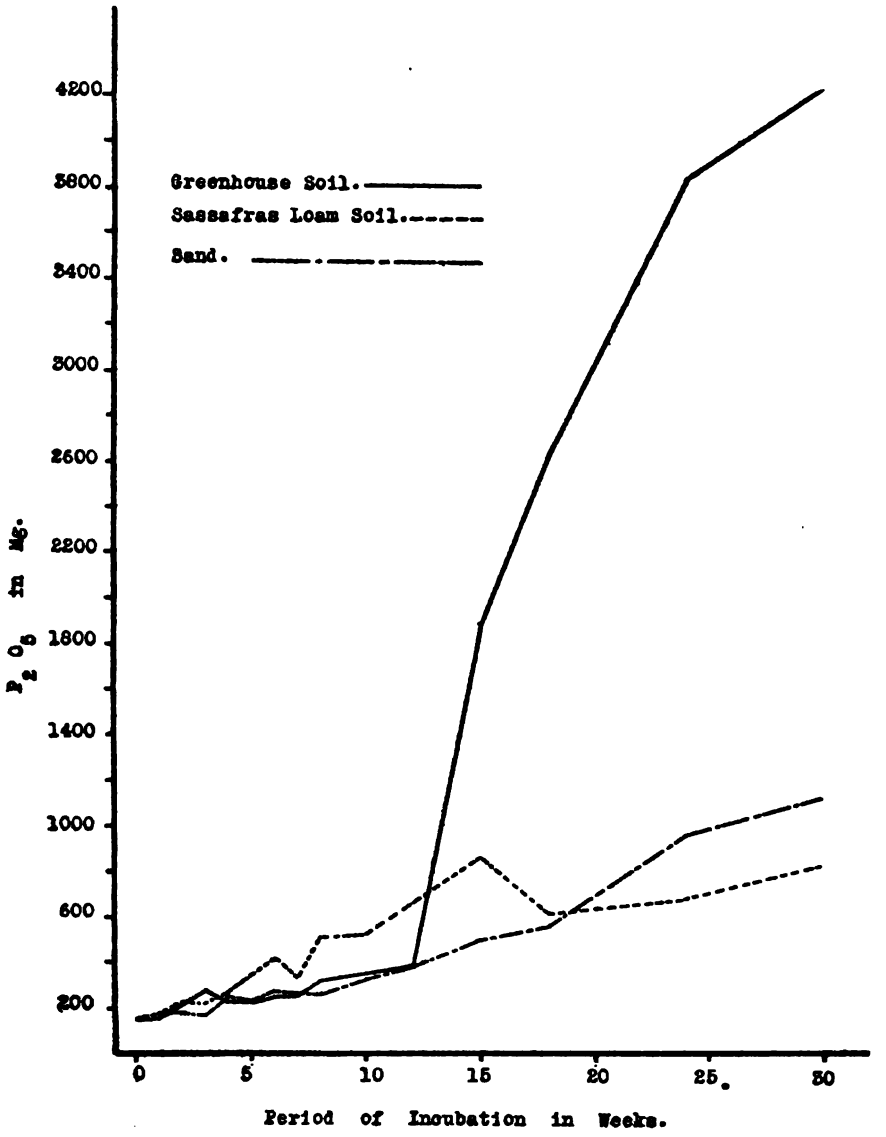

Fig. 1.—Curves showing the citrate-soluble phosphoric acid in the composts during a period of 30 weeks.

174.50 c.c., respectively. At the end of the eighteenth week the corresponding figures were 100.00 c.c. and 414.00 c.c., respectively. Be-

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tween the eighteenth and the end of the twenty-fourth week the acidity equivalent of the sulfur-floats-sand mixtures increased from 414.00 c.c. to 932.00 c.c. It would seem, therefore, that the sulfofication process gained in intensity most markedly after the eighteenth week. This assumption is supported by the data representing the amounts of citrate-soluble phosphoric acid found at the end of the different periods.

Comparing the figures for citrate-soluble phosphoric acid in the floats-sand and sulfur-floats-sand mixtures, one may note relatively small differences for the first three weeks. Beyond that period the differences become marked in favor of the sulfur-floats-sand mixtures. After the fifth week there was a decrease in the amounts of citrate-soluble phosphoric acid in the floats-sand mixtures, whereas in the sulfur-floats-sand mixtures there was, with one exception, a constant increase. It will be observed, for instance, that at the end of the eighteenth week there was found in the floats-sand mixture an equivalent of 140.20 mg. of citrate-soluble phosphoric acid, while in the sulfur-floats-sand mixture the corresponding amount was 455.95 mg. At the end of the twenty-fourth week the corresponding figures were 127.80 mg. and 949.07mg., respectively; and at the end of the thirty weeks, 129.30 mg. and 1115.10 mg., respectively. There can be no doubt therefore, that, notwithstanding the poverty of the sand as a culture medium, a large proportion of the sulfur added was oxidized to sulfuric acid, and that the latter readily reacted with the tricalcic phosphate to form citrate-soluble phosphate.

As a culture medium the greenhouse soil was much superior to the sand. It was originally rich in nitrogen and mineral plant-food constituents and had been still further enriched by additions of organic matter. The latter had undergone more or less thorough decomposition prior to the use of the soil in the experiments described here. Hence the sulfofying bacteria should have accomplished a greater amount of change in the greenhouse soil than they did in the sand, as is indeed borne out by the data presented in Table II.

It was to be expected that the soil portions which received no addition of either sulfur or floats would show no appreciable increase in acidity or available phosphoric acid. Such was really the case as indicated by the figures in columns 1 and 2 of Table II. There was, as a matter of fact, a slight decrease in the amount of available phosphoric acid, brought about, probably, by the assimilation of some of the available phosphorus by the microörganisms in the soil. On the other hand, in the sulfur-soil mixtures there was a steady and marked increase in the acidity equivalents until the end of the seventh week. At that time the amount of acid found was equivalent to 310.60 c.c. of N/50 KOH. But from the end of the seventh to the end of the tenth week there was a most remarkable increase in the acidity equivalent. From an acidity equivalent of 310.60 c.c. there was an increase to an acidity equivalent of 4404.00 c.c. of N/50 KOH. After that there was a slight,

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but nevertheless, marked increase until the end of the eighteenth week. Corresponding soil portions were not available for the determination of the acidity equivalents at the end of the twenty-fourth and thirtieth weeks. With the accumulation of free acid and of acid salts in the soil portions there was also an increase in the amount of available phosphoric acid. Evidently the phosphorus compounds in the soil were acted upon by the sulfuric acid and the amount of available phosphoric acid increased from 105.18 mg. to a maximum of 160.10 mg. at the end of the eighteenth week.

In the floats-soil mixtures there was no perceptible accumulation of free acid or of available phosphates. In the absence of sulfur this could not have taken place. But in the sulfur-floats-soil mixtures the results were quite different. Here the sulfur led to an accumulation of acid which, for a time, was greater in quantity than in the corresponding sulfur-soil portions. It will be noted, for instance, that at the end of the fifth week the acidity equivalents in the sulfur-soil and sulfur-floats-soil mixtures were 137.00 c.c., and 209.00 c.c., respectively. However, after the eighth week the increase in the acidity equivalents was much slighter in the sulfur-floats-soil mixtures. At the end of the tenth week the acidity equivalent of the latter was only 646.00 c.c. as against 4404.00 c.c. in the sulfur-soil mixtures. A greater increase in the acidity equivalents of the sulfur-floats-soil mixtures occurred between the eighteenth and the twenty-fourth weeks; nevertheless, the maximum accumulation of acid was never as great as it was in the corresponding sulfur-soil mixtures.

It must be concluded, therefore that much of the acid which resulted from the oxidation of the sulfur in the sulfur-floats-soil mixtures had reacted with the tricalcic phosphate and made its phosphorus available as measured by the method of the Official Agricultural Chemists. Proof of this assumption is furnished in the last column of Table II. It is shown there that the accumulation of available phosphoric acid proceeded at first rather slowly. At the end of the twelfth week the amount of available phosphoric acid was equivalent to only 396.88 mg. After that time, however, the increase was very rapid, for the available phosphoric acid was equivalent to 1982.40 mg. at the end of the fifteenth week, 2605.62 mg. at the end of the eighteenth week, 3840.90 mg. at the end of the twenty-fourth week and 4126.48 mg. at the end of the thirtieth week.

The amounts of available phosphoric acid shown to have been present by the official method were probably below the true value, since some of the phosphoric acid had entered into combination with iron and aluminuim compounds whose availability to growing crops may be greater than that indicated by the official method. As it is, more than 85 per cent of the phosphoric acid present in the 15 gm. of floats had become available. Hence it is safe to assume that in suitable soil cul-

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ture media large quantities of sulfur may be oxidized by microörganisms; and that the sulfuric acid accumulating in the soil may react with insoluble tricalcic phosphates. That the nature of the medium is one of the controlling factors is readily seen from a comparison of the sand and soil mixtures, respectively, to which both sulfur and floats had been added. The data in question which may be found in the last columns of Table I and II show that at the end of the twelfth week the sand mixtures contained 390.62 mg. of available phosphoric acid while the corresponding greenhouse soil mixtures contained 396.88 mg. But at the end of the fifteenth week the corresponding figures were 400.68 mg. and 1982.40 mg.,respectively; at the end of the twenty-fourth week, 949.07 mg. and 3840.90 mg., respectively; and at the end of the thirtieth week 1115.10 mg. and 4126.48 mg., respectively.

In Table III are given the results obtained in the Sassafras loam soil. These results differ in degree rather than in kind, from those already recorded for the sand and for the greenhouse soils. A brief survey of the data should therefore suffice to confirm those already discussed. The same absence of acid and of available phosphorus accumulation in the soil (alone) portion, and the same marked acid accumulation in the sulfur-soil portions may be noted. In the case of the sassafras soil the acid accumulation was not as great as in the greenhouse soil, but much greater than in the sand. Similarly there was no accumulation of available phosphoric acid in the floats-soil portions. Curiously enough, the accumulation of available phosphoric acid in the sulfur-floats-soil mixtures was greater in the sassafras soil at the end of the tenth week than it was in the corresponding sand and greenhouse soil portions. Subsequently, however, both the sand and greenhouse soil portions showed a great accumulation of available phosphoric acid. Thus at the end of thirty weeks the figures for the sand, greenhouse soil and sassafras soil were 1115.10 mg., 4126.48 mg., and 822.70 mg., respectively. There is no doubt that the failure of the sassafras soil to accumulate larger quantities of available phosphoric acid should be ascribed partly to the less favorable conditions which it offered for rapid oxidation. It is possible, also, that the chemical constitution of this soil may have had something to do with the differences noted. It will be observed, further, that in the soil portions in which precipitated sulfur was used in the place of flowers of sulfur the results obtained were very much like those already considered. They serve to show that the biological changes were limited in a rather definite way by the physical and chemical properties of the medium. They also serve to confirm the accuracy of the analytical determinations.

It may not be out of place here to point out again that the wide variation in the composition of the soils employed in these experiments and the consequent differences in their absorptive power must have

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played a part in the accumulation both of acid and of available phosphoric acid. As has been shown by Fraps (17) the absorptive power of soils is closely related to their iron and aluminum content. It is probable that a greater proportion of the phosphorus of the floats was made available than is actually shown by the ammonium citrate extractions.

The absorptive power of the sand and the two soils was determined by adding to 50 gm. of each, 100 c.c. of a solution containing 300 mg. of P_2O_5 in the form of potassium phosphate. After one week the solutions were filtered off from the soil and the amount of phosphorus still in solution determined. From these determinations the amount of P_2O_5 fixed per gram of soil was calculated. The results are as follows:

Sand	0.354 mg. P_2O_5 fixed per gram
Greenhouse soil	4.000 mg. P_2O_5 fixed per gram
Loam soil	2.650 mg. P_2O_5 fixed per gram

From the results of the fixation tests it can be seen that the greenhouse soil was able to fix about double the amount of P_2O_5 fixed in the Sassafras loam soil. It must be assumed therefore, that a portion of the phosphoric acid liberated from the floats was again made insoluble by combining with the iron and aluminum present. The amounts of available phosphoric acid shown by the ammonium citrate extractions must therefore differ, somewhat, from the amounts actually made available.

The Accumulation of Water-Soluble Phosphoric Acid

The water-soluble phosphoric acid found in the different soil mixtures was determined and its amount recorded in Table IV.

The results recorded in Table IV are the averages of duplicate determinations. It will be noted that the content of water-soluble phosphoric acid increased in all of the mixtures which had received additions of both sulfur and floats. A slight increase is shown in the sassafras and greenhouse soil portions which had received additions of floats alone, but this increase is within the limits of experimental error.

At the end of the eighth week the sulfur-floats-sand mixtures contained 100.95 mg. of water-soluble phosphoric acid. The corresponding amounts in the greenhouse and sassafras soils were 54.71 mg. and 128.15 mg., respectively. It seems, therefore, that whatever phosphate had been made water-soluble in the greenhouse soil by the oxidized sulfur was changed again into water-insoluble forms. The same relations appear at the end of the tenth and twelfth weeks. At the end of the fifteenth week the content of water-soluble phosphoric acid in the sulfur-floats-sand, sulfur-floats-greenhouse soil and the sulfur-floats-sassafras soil was 185.23 mg., 999.56 mg. and 178.11 mg., respectively. It is evident that the greenhouse soil had gained in a very striking way on the other

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TABLE IV

THE CONTENT OF WATER-SOLUBLE PHOSPHORIC ACID

	Beginning P_2O_5, mg.	End of 8th Week P_2O_5, mg.	End of 10th Week P_2O_5, mg.	End of 12th Week P_2O_5, mg.	End of 15th Week P_2O_5, mg.	End of 18th Week P_2O_5, mg.	End of 24th Week P_2O_5, mg.	End of 30th Week P_2O_5, mg.	Increase Due to Sulfur P_2O_5, mg.
100 gm. Sand, 15 gm. Floats	17.72	9.07	12.39	.....	15.10	14.10	.....	.....	.....
100 gm. Sand, 15 gm. Floats, 5 gm. Flowers of Sulfur	17.09	100.95	156.02	174.03	185.23	230.45	473.30	557.55	540.46
100 gm. Greenhouse Soil, 15 gm. Floats	21.28	32.64	30.38	.....	30.10	28.78	.....	.....	.....
100 gm. Greenhouse Soil, 15 gm. Floats, 5 gm. Flowers of Sulfur	19.91	54.71	44.58	55.71	999.56	1295.99	1234.04	1437.24	1417.33
100 gm. Sandy Loam Soil, 15 gm. Floats	20.35	10.27	25.24	.....	18.40	20.13	.....	.....	.....
100 gm. Sandy Loam Soil, 15 gm. Floats, 5 gm. Flowers of Sulfur	20.97	128.15	129.97	136.78	178.11	251.88	215.23	224.26	203.29
100 gm. Sandy Loam Soil, 15 gm. Floats, 5 gm. Precipitated Sulfur	17.52	128.76	121.20	113.93	118.32	200.46	218.94	223.02	205.50

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soils. The differences are even more striking at the end of the eighteenth and thirtieth weeks. At the end of the twenty-fourth week the greenhouse soil failed for some reason to show an increase over the eighteenth week. On the whole, the data at hand show an interesting accumulation of water-soluble phosphoric acid in the sand and greenhouse soils at the end of the thirtieth week. A comparison of Table IV with Tables I and II will show that in the sand more than half of the available phosphoric acid was present in water-soluble form at the end of the thirty weeks. In the greenhouse soil more than a third of the available phosphoric acid was water-soluble at the end of thirty weeks. In this instance, again, the mechanical and chemical nature of the soil media employed served to determine the extent of absorption and adsorption, and hence the proportion of citrate-soluble and water-soluble phosphoric acid. As to the relative influence of the flowers of sulfur and precipitated sulfur used with the sassafras soil, the determinations agree fairly well and do not show that the precipitated sulfur was superior to the flowers of sulfur.

The Oxidation of Sulfur as Measured in Terms of Sulfates

The determination of the yields of sulfates in the various soil mixtures shows clearly to what extent the sulfur was oxidized at the different periods. It will be noted from the data shown in Table V that, in the sand to which sulfur alone was added, the initial sulfur content, expressed in terms of SO_4, was 6 mg. At the end of the fifth week, the corresponding sulfur content was 15.56 mg. A gradual increase occurred to the end of the eighteenth week. No determinations were made after that period in the sulfur-sand mixtures for lack of material.

We note, further, that, in the sand to which both sulfur and floats were added, the oxidation of the sulfur was much more rapid than it was in the sulfur-sand mixtures. Thus, at the end of the fifth week, the sulfur-floats-sand mixtures contained 612.39 mg. of SO_4 as against only 15.56 mg. in the sulfur-sand mixtures. At the end of the fifteenth week, the corresponding figures were 908.64 as against 74.33, and, at the end of the eighteenth week, 1102.08 as against 100.25. At the end of the thirtieth week, there were found in the sulfur-floats-sand mixtures 2603.62 mg. of SO_4, equivalent to 816.78 mg. of sulfur. It is clear, therefore, that the presence of tricalcium phosphate hastened the oxidation of the sulfur, probably for two reasons. It may be assumed that the microörganisms responsible for the oxidation of the sulfur require more or less phosphorus for their life activities. It may be assumed, also, that, as the sulfur is oxidized to sulfuric acid, the latter may accumulate in sufficient quantity to make the medium too acid for the proper development of the microörganisms. By neutralizing some of the free acid, the tricalcic phosphate reduces the acidity of the medium and makes possible thereby the continued growth of the sulfur-oxidizing

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organisms. That this assumption is justified is borne out by the figures given in Tables I, II and III, showing the amounts of phosphorus available according to the methods of the Official Agricultural Chemists. The data in question are again recorded in Table V. It is shown there that the phosphoric acid soluble in ammonium citrate was equivalent in the sulfur-floats-sand mixtures to 85.78 mg. at the end of the fifth week,

TABLE V

THE CONTENT OF SULFATES IN THE DIFFERENT MIXTURES

Time	Additions: 5 gm. Sulfur — P_2O_5 Soluble in Ammonium Citrate mg.	Sulfates Soluble in 1% HCl — Average mg. of SO_4	Sulfates Soluble in 1% HCl — Inc. over Check mg. of SO_4	Amount of Sulfur Oxidized mg. of S	5 gm. Sulfur, 15 gm. Floats — P_2O_5 Soluble in Ammonium Citrate Inc. over Check mg.	Sulfates Soluble in 1% HCl — Average mg. of SO_4	Sulfates Soluble in 1% HCl — Inc. over Check mg. of SO_4	Amount of Sulfur Oxidized mg. of S
SAND								
Beginning	35.06	6.00	.....	.....	.....	156.70	.....	.....
End of 5th week..	28.23	15.56	9.56	3.19	85.78	612.39	455.69	152.11
End of 7th week..	24.18	67.41	61.41	20.50	119.69	699.31	542.61	181.12
End of 15th week..	33.80	74.33	68.33	22.81	261.64	908.64	851.94	284.36
End of 18th week..	28.90	100.25	94.25	31.46	316.91	1102.08	945.38	315.57
End of 24th week..	.....	.....	.....	.....	810.03	2431.78	2275.08	759.42
End of 30th week..	.....	.....	.....	.....	976.06	2603.62	2446.92	816.78
GREENHOUSE SOIL								
Beginning	105.18	121.10	.....	.....	.....	152.16	.....	.....
End of 5th week..	100.34	981.79	860.69	287.27	88.26	1424.30	1272.14	424.64
End of 7th week..	103.49	1559.12	1438.02	480.01	111.23	2007.03	1854.87	619.13
End of 15th week..	160.10	5294.16	5173.06	1727.75	1839.74	5744.64	5592.48	1866.78
End of 18th week..	150.20	5873.14	5752.04	1920.02	2462.96	8559.60	8407.44	2806.40
End of 24th week..	.....	.....	.....	.....	3698.24	9213.60	9061.44	3024.71
End of 30th week..	.....	.....	.....	.....	3983.82	10524.96	10372.80	3462.44
SASSAFRAS LOAM								
Beginning	83.42	130.25	.....	.....	.....	168.10	.....	.....
End of 5th week..	103.97	708.54	578.29	193.03	187.88	1035.36	867.26	289.49
End of 7th week..	89.47	751.88	621.63	207.49	185.46	993.70	825.60	275.59
End of 15th week..	101.40	1116.61	986.36	329.25	713.03	2706.24	2538.14	847.23
End of 18th week..	100.00	1381.11	1250.86	417.52	457.80	2027.04	1858.94	620.50
End of 24th week..	.....	.....	.....	.....	534.61	2224.37	2056.27	686.38
End of 30th week..	.....	.....	.....	.....	668.43	2477.18	2309.08	770.77

to 261.64 mg. at the end of the fifteenth week, to 810.03 mg. at the end of the twenty-fourth week, and to 976.06 mg. at the end of the thirtieth week.

In the greenhouse soil mixtures, the corresponding reactions were very similar in character. Because of its more favorable character as a medium for the growth of the microörganisms, the oxidation of the

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sulfur proceeded more rapidly in it. We note, thus, that the content of sulfates, expressed in terms of SO_4, in the sulfur-soil mixtures was 981.99 mg. at the end of the fifth week, 5294.16 mg. at the end of the fifteenth week, and 5873.14 mg. at the end of the eighteenth week. In the corresponding sulfur-floats-soil mixture, the oxidation of the sulfur was again more rapid than it was in the sulfur-soil mixtures, as was already noted in the case of the sand medium. For instance, at the end of the fifth week, the sulfur-floats-soil mixtures contained an equivalent of 1424.30 mg. of SO_4 as against 981.79 mg. of SO_4 in the sulfur-soil mixtures. Similar differences were found at the end of the other

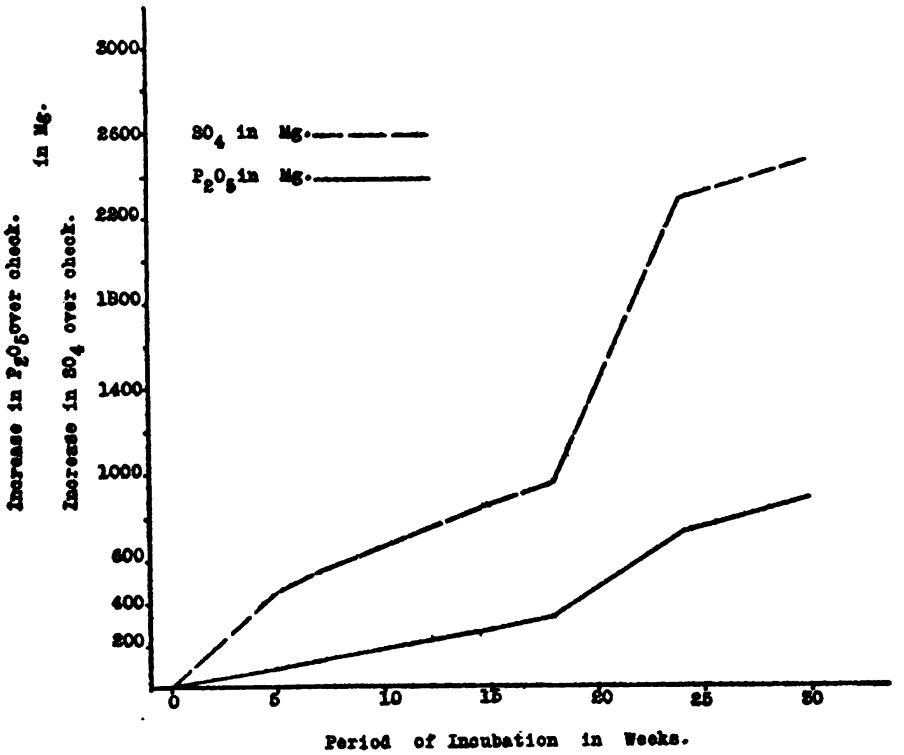

Fig. 2.—Curves showing the citrate-soluble P_2O_5 and SO_4 found in the composts made up with the sand during a period of 30 weeks.

periods. It may be noted particularly that, at the end of the thirtieth week, the sulfur-floats-soil mixtures contained an equivalent of 10,524.96 mg. of SO_4, which would correspond to 3462.44 mg. of sulfur. It seems, therefore, that, in the greenhouse soil, nearly 3.5 gm. of sulfur were oxidized to sulfates out of a total of 5 gm. of sulfur applied. This change was accomplished in the mixtures containing both floats and sulfur and in a period of thirty weeks. As the amount of oxidized sulfur increased in these mixtures, the amount of available phosphorus also increased. This should have been expected, since, as already noted in the case of the sand mixtures, the accumulated sulfuric acid would

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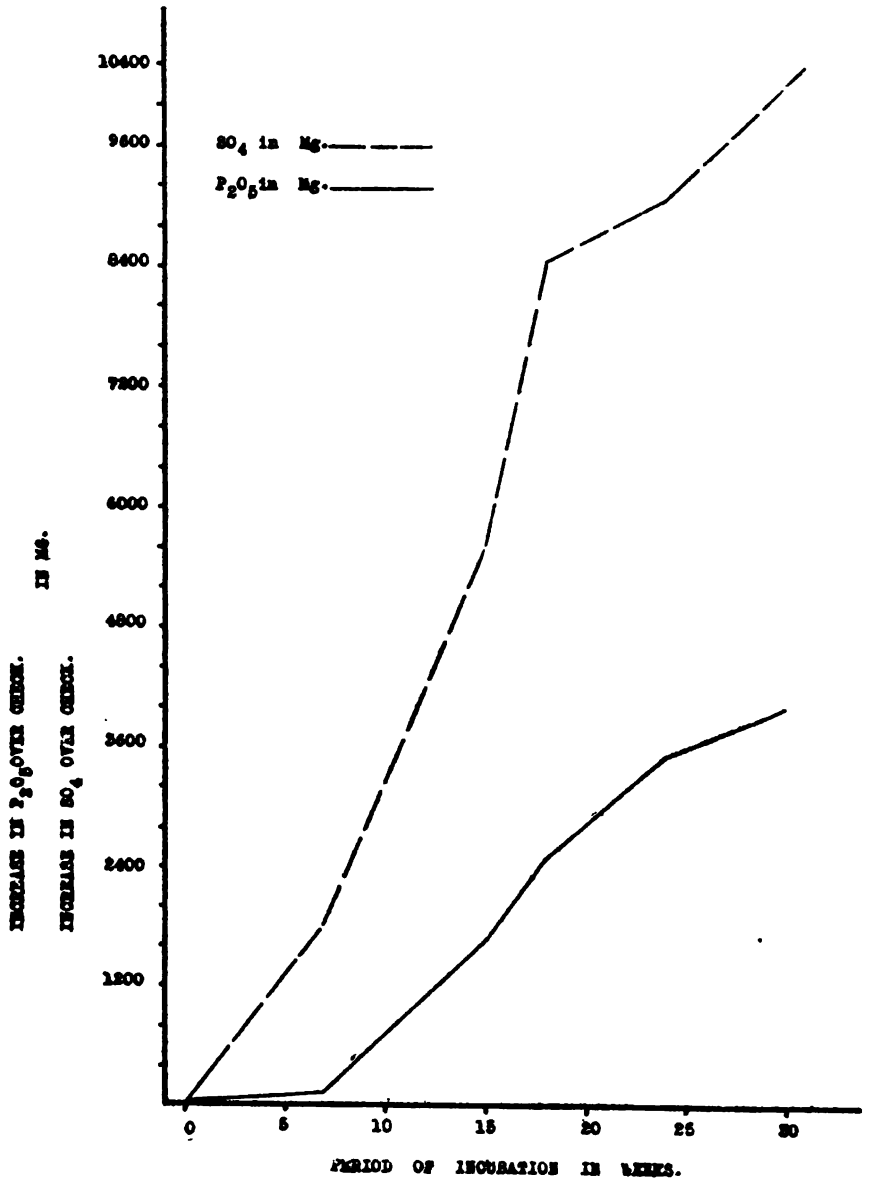

Fig. 3.—Curves showing the citrate-soluble P_2O_5 and SO_4 found in the composts made up with greenhouse soil during a period of 30 weeks.

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tend to react with the tricalcic phosphate and to make it available according to the official method.

In the case of the Sassafras loam soil, we observe the gradual oxidation of a portion of the sulfur used in the sulfur-soil mixtures, the more intense oxidation of sulfur used in the sulfur-floats-soil mixtures, the accumulation of sulfates and also of citrate-soluble phosphates. It will be readily seen, at the same time, that the Sassafras loam was not as good a medium for the activities of the sulfur-oxidizing organisms as

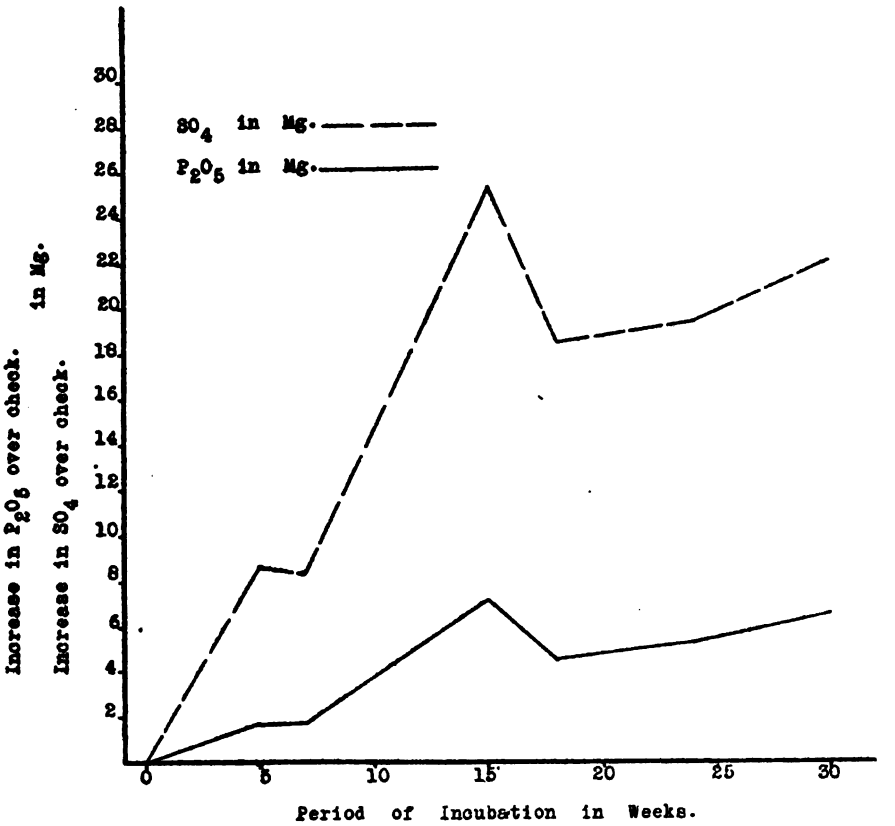

Fig. 4.—Curves showing the citrate-soluble P_2O_5 and SO_4 found in the composts made up with Sassafras loam soil during a period of 30 weeks.

was the greenhouse soil. In fact, it was inferior to the sand as a culture medium during the latter periods of the experiment, for, at the end of the thirtieth week, the accumulation of sulfates and of citrate-soluble phosphates was slighter in the sulfur-floats-Sassafras loam mixtures than it was in the corresponding sulfur-floats-sand mixtures. The inferiority of the Sassafras loam as a culture medium must be ascribed to its less favorable mechanical composition as well as to the possible accumulation of acid salts of iron and alumina that may have exerted a toxic effect on the microörganisms.

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In concluding the consideration of the data contained in Table V, mention should be made of the ratio of oxidized sulfur to available phosphoric acid. It will be observed that, in the sulfur-floats-sand or sulfur-floats-soil mixtures, the quantities of P_2O_5, expressed in milligrams, closely correspond to the quantities of sulfur expressed in milligrams. Thus, in the sand mixtures there were found at the end of the fifteenth week 261.64 mg. of P_2O_5 and 284.36 mg. of oxidized sulfur. In the corresponding greenhouse soil mixtures there were found at the end of the fifteenth week 1839.74 mg. of citrate-soluble P_2O_5 and 1866.78 mg. of sulfur in the form of sulfates. In the corresponding Sassafras loam soil mixtures there were found at the end of the fifteenth week 713.03 mg. of phosphoric acid and 847.23 mg. of sulfur. It would appear, therefore, that, under the conditions of the experiment, the oxidation of one part of sulfur made available an equivalent of one part of P_2O_5. The significance of this relation is obvious when considered from the standpoint of the composting of floats with sulfur for the purpose of making the phosphorus in the former more readily available to growing vegetation.

The Formation of Available Phosphoric Acid in Constant Quantities of Soil with a Varying Content of Sulfur and Floats

In order that the phosphorus in floats may be made available most advantageously under farm conditions, suitable proportions of soil, moisture, sulfur, floats and organic matter must be employed. In so far as the sulfur and floats are concerned, concentrated composts would be, everything else being equal, more economical than less concentrated composts. If, let us say, 1000 pounds of floats could be treated effectively in a one-ton mixture, the cost of the treatment would be less than if the same quantity of floats were rendered available in a two or three-ton mixture. It was proposed, in outlining the experiment described below, to determine, if possible, the best proportions of sulfur to floats. Varying quantities of these ingredients were mixed with 100-gm. quantities of Sassafras loam soil. The mixtures were maintained at the moisture content of about 24 per cent. At the end of six weeks and of twelve weeks, respectively, the acidity and ammonium citrate and water-soluble phosphoric acid were determined. The arrangement of the experiment and the results secured are shown in Table VI.

It will be observed that 15 gm. of floats were mixed with sulfur varying in quantity from 1 gm. to 15 gm. and that 5 gm. of sulfur were mixed with floats varying in amount from 3 gm. to 30 gm. The results indicate that at the end of six weeks practically maximum amounts of available phosphoric acid were obtained from the 15 gm. of floats used with 1 gm. of sulfur. The actual maximum, both of available phosphoric acid and of acidity, was obtained when the 15 gm. of floats were used together with 9 gm. of sulfur. At the end of twelve weeks,

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the maximum amount of available phosphoric acid was obtained from 15 gm. of floats when the latter were used together with 9 gm. of sulfur. On the other hand, the maximum degree of acidity was developed when 15 gm. of floats were used together with 7 gm. of sulfur.

With the quantity of sulfur kept constant, viz: 5 gm., and the quantity of floats variable, the maximum quantity of available phos-

TABLE VI

INFLUENCE OF VARYING PROPORTIONS OF SULFUR AND FLOATS ON ACCUMULATION OF AVAILABLE PHOSPHORIC ACID

Lab. No and Additions	At End of Six Weeks						At End of Twelve Weeks			
		Ammonium Citrate-Soluble P_2O_5			Water Soluble P_2O_5			Ammonium Citrate-Soluble P_2O_5		
	Acidity, c.c. of N/50 KOH	Mg.	Increase over Check, mg.	Increase per gm. of Floats, mg.	Mg.	Increase over Check, mg.	Acidity, c.c. of N/50 KOH	Mg.	Increase over Check, mg.	Increase per gm. of Floats, mg.
1. None	7.50	99.14	.....	.....	13.20	.....	7.90	100.40	.....	.....
2. 15 gm. Floats....	6.50	155.75	.....	.....	18.75	.....	7.95	150.00	.....	.....
3. 15 gm. Floats, 1 gm. Sulfur	400.00	233.88	78.13	5.21	30.47	11.72	520.00	310.99	160.99	10.73
4. 15 gm. Floats, 2 gm. Sulfur	436.00	239.92	84.17	5.61	31.71	12.96	484.00	311.48	161.48	10.77
5. 15 gm. Floats, 3 gm. Sulfur	412.00	226.94	71.19	4.75	30.23	11.48	500.00	312.23	162.23	10.81
6. 15 gm. Floats, 5 gm. Sulfur	408.00	238.83	83.08	5.54	33.20	14.45	498.00	314.71	164.71	10.98
7. 15 gm. Floats, 7 gm. Sulfur	456.00	252.71	96.96	6.46	35.18	16.43	676.00	410.60	260.60	17.37
8. 15 gm. Floats, 9 gm. Sulfur	456.00	258.65	102.90	6.86	29.23	10.48	564.00	480.73	330.73	22.05
9. 15 gm. Floats, 11 gm. Sulfur	392.00	238.83	83.08	5.54	30.23	11.48	466.00	329.57	179.57	11.97
10. 15 gm. Floats, 15 gm. Sulfur	448.00	243.80	88.05	5.87	30.72	11.97	588.00	370.46	220.46	14.69
11. 3 gm. Floats, 5 gm. Sulfur	432.00	137.75	27.30	9.10	19.23	4.92	552.00	142.98	32.66	10.89
12. 5 gm. Floats, 5 gm. Sulfur	404.00	176.39	58.45	11.69	19.82	4.77	658.00	340.73	223.80	44.76
13. 8 gm. Floats, 5 gm. Sulfur	360.00	189.28	59.98	7.49	22.55	5.39	496.00	236.65	109.80	13.72
14. 12 gm. Floats, 5 gm. Sulfur	392.00	196.22	51.84	4.32	28.00	10.36	508.00	276.30	136.23	11.35
15. 20 gm. Floats, 5 gm. Sulfur	444.00	284.43	109.89	5.49	37.30	16.70	550.00	365.51	198.99	9.95
16. 30 gm. Floats, 5 gm. sulfur	496.00	346.85	134.61	4.49	34.93	10.63	520.00	432.41	232.83	7.76

phoric acid was secured from the largest amount of floats. This was true of both the six and twelve week periods. It would seem, all told, that, under favorable conditions, one part of sulfur and two parts of floats may constitute a satisfactory combination for the production of available phosphoric acid. This conclusion is at present tentative, in view of the somewhat conflicting data and the short period of the experiment. It is

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strengthened, however, by the results already recorded in Tables I to IV. It should be added here that, sulfur being the most expensive of the constituents, it would be desirable to use as wide a ratio of floats to sulfur as may be consistent with the greatest yield of available phosphoric acid.

Moisture as a Factor in Sulfur Oxidation

The importance of the moisture factor in the oxidation of sulfur by microörganisms became evident during the progress of the experiments described in the foregoing pages. For this reason it seemed desirable to make a more careful study of the influence of varying amounts of moisture on the oxidation of sulfur. One hundred-gm. portions of the Sassafras loam soil were used and the mixtures were made up in the same manner as those already described. "Optimum Moisture" as used in this experiment is 50 per cent of the water-holding capacity of the soil mixtures. The optimum moisture for the soil-floats mixtures was 24.25 c.c. and for the soil-floats-sulfur mixtures 24.00 c.c. After incubation for 6 weeks the mixtures were examined for acidity and for ammonium citrate and water-soluble phosphoric acid. The plan of the experiment and the results obtained are recorded in Table VII, and are shown graphically in figure 5.

The results presented in Table VII show that the moisture content has an important influence on the processes in which sulfur undergoing oxidation, renders the phosphoric acid of the floats available. However, where there was no sulfur to be oxidized, as was the case where floats only were used, no distinctive differences are noted. These statements hold true for both citrate and water-soluble phosphorus. Another fact which is clearly brought out for the soil-floats-sulfur mixtures is that a low and a high moisture content retards the production of citrate and water-soluble phosphorus.

A more detailed study of the results obtained with the soil-floats mixtures show that portions 5 and 6 which received 1¼ and 1½ optimum moisture, respectively, contained slightly less available phosphoric acid than the others. This is due either to more thorough fixation or to the reversion of the soluble phosphoric acid originally present in the floats. It is possible, however, that the large proportion of moisture affected somewhat the ammonium citrate determinations.

With the soil-floats-sulfur mixtures the acidity increased from 36 c.c., expressed as N/50 KOH where ¼ optimum moisture was added, to 484 c.c. with an addition of moisture equivalent to ¾ optimum. A further addition of moisture slightly decreased the acidity, but when 1½ optimum moisture was added there was a large decrease. The acidity in this case was equivalent to 60 c.c. N/50 KOH.

A comparison of the amounts of citrate-soluble phosphoric acid in the soil-floats-sulfur mixtures will show that the increase was from 165.26

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mg. in the composts receiving ¼ optimum, to 289.21 mg. of P_2O_5 in the portions receiving 1¼ optimum moisture. It seems, therefore, that 1¼ optimum moisture conditions are the best in this soil for maximum results, in rendering available insoluble phosphates such as raw rock phosphate.

The results apparently show that the maximum oxidation of sulfur takes place under ¾ optimum moisture conditions, as is shown by the acidity, but the conditions for the greatest solvent effect on the rock phosphate are found in the soils with a high moisture content. The reversion of some of the phosphorus which had been made available may be offered as a possible explanation of the relations just noted.

TABLE VII

THE INFLUENCE OF MOISTURE ON THE ACCUMULATION OF ACIDITY AND OF AVAILABLE PHOSPHORIC ACID

Lab. No. and Additions	Moisture Proportion of Optimum	H_2O, c.c	At End of Six Weeks				At End of Twelve Weeks			
			Acidity, c.c. of N/50 KOH	Citrate-Soluble P_2O_5 in Composts		Water-Soluble P_2O_5 in Composts, mg.	Acidity, c.c. of N/50 KOH	Citrate-Soluble P_2O_5 in Composts		Water-Soluble P_2O_5 in Composts, mg.
				P_2O_5, mg.	Increase over Check, mg.			P_2O_5, mg.	Increase over Check, mg.	
1. 15 gm. Floats.....	¼	6.10	10.00	172.77	.....	18.09	9.80	170.00	.....	12.60
2. 15 gm. Floats.....	½	12.15	14.00	176.53	.....	13.63	9.30	170.80	.....	12.60
3. 15 gm. Floats.....	¾	18.20	7.00	170.27	.....	16.60	10.00	172.80	.....	14.40
4. 15 gm. Floats.....	1	24.25	14.00	172.77	.....	14.12	9.50	168.10	.....	12.80
5. 15 gm. Floats.....	1¼	30.35	11.00	169.02	.....	15.86	10.10	170.00	.....	12.40
6. 15 gm. Floats.....	1½	36.40	20.00	167.76	.....	17.59	9.60	167.90	.....	13.68
7. 15 gm. Floats, 5 gm. Sulfur...	¼	6.00	36.00	165.26	—7.51	15.86	200.00	230.10	60.10	25.90
8. 15 gm. Floats, 5 gm. Sulfur...	½	12.00	32.00	179.03	2.50	16.85	278.40	320.00	149.20	46.70
9. 15 gm. Floats, 5 gm. Sulfur...	¾	18.00	484.00	256.03	85.76	27.65	495.20	500.00	327.20	118.20
10. 15 gm. Floats, 5 gm. Sulfur...	1	24.00	448.00	282.95	110.18	31.22	580.40	580.40	412.30	126.00
11. 15 gm. Floats, 5 gm. Sulfur...	1¼	30.00	440.00	289.21	120.19	31.22	651.00	620.30	450.30	131.40
12. 15 gm. Floats, 5 gm. Sulfur...	1½	36.00	60.00	190.30	22.54	14.87	400.10	390.00	222.10	49.80

The amount of water-soluble phosphoric acid in the composts is not large, but the results show that optimum and 1¼ optimum moisture conditions give the best results.

The results secured at the end of 12 weeks confirm, in most respects, those secured at the end of the shorter period. It will be observed, however, that the maximum accumulation of acid occurred here with 1¼ optimum moisture content. The maximum accumulation of citrate and water-soluble phosphoric acid occurred with the same moisture content. When the moisture content was increased beyond 1¼ optimum the accumulation of acidity and of available phosphoric acid was depressed.

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Biological Factors

The results already recorded naturally raise the question as to the nature of the biological factors involved in the oxidation of sulfur to sulfuric acid. Other investigators have apparently demonstrated that the oxidation of sulfur in soils is a biological process. Its seems desirable, nevertheless, to determine in the present experiment the extent to which biological factors had to do with the oxidation of sulfur. To this end, 100-gm, portions of Sassafras loam soil were arranged in three

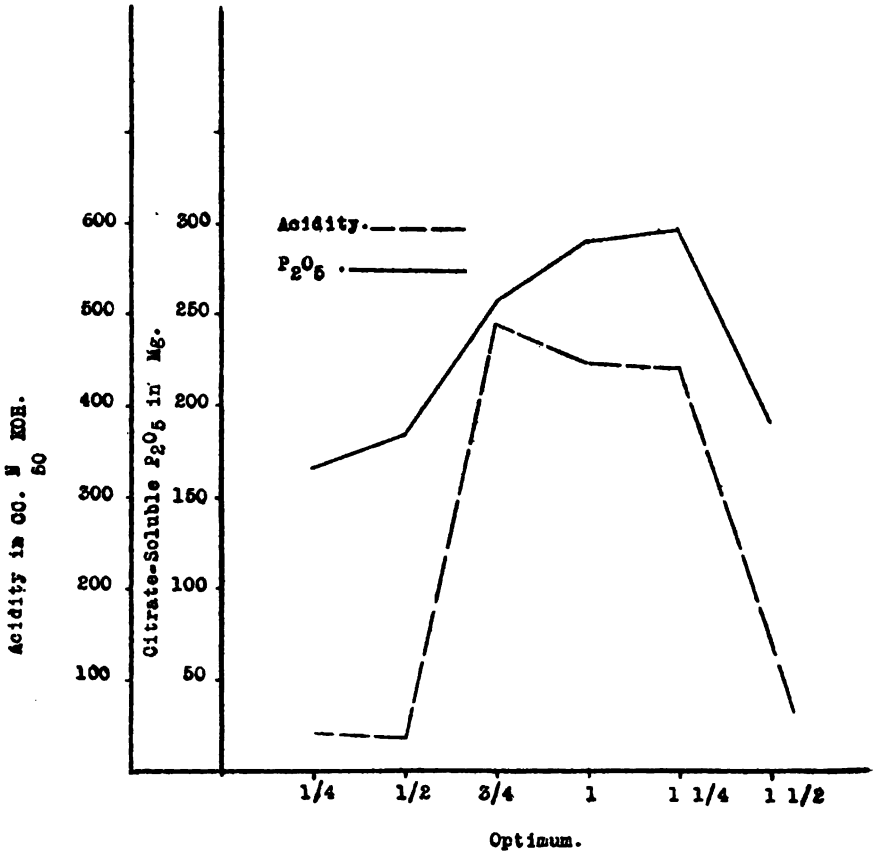

Fig. 5.—Curves showing the influence of moisture content on the availability of phosphorous and acidity: length of incubation period 6 weeks.

groups, designated, respectively, as (1) Untreated (2) Sterilized and Inoculated (3) Unsterilized and Inoculated. The inoculation was effected by means of a soil infusion derived from soil supposedly well provided with sulfur-oxidizing organisms. In making the inoculation, 10-gm. portions of soil were shaken with 100 c.c. of water, and 5 c.c. of the infusion thus prepared were added to each 100-gm portion of the Sassafras loam soil. The soil to be sterilized was autoclaved at 15 pounds for 15 minutes. The arrangement of the series in question, together

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with the results secured at the end of 7 and 14 weeks, respectively, are shown in Table VIII.

In drawing any conclusions from the results reported above, it appears necessary to accredit the soil infusion with bringing about an increase in the amount of available phosphorus. Thus, the sulfur-floats-soil mixture, which contained unsterilized and inoculated soil, contained, at the end of 7 weeks, 434.44 mg. of phosphoric acid, and, at the end of 14 weeks, 634.37 mg. of phosphoric acid as against corresponding quantities of 250.42 mg. of phosporic acid and 339.48 mg. of phosphoric acid, respectively, for the corresponding portions which were unsterilized but not inoculated. Other experiments, both with crude and pure cultures of soil microörganisms, are now in progress for the purpose of securing additional information on this point.

TABLE VIII

INFLUENCE OF BIOLOGICAL FACTORS ON THE AVAILABILITY OF THE PHOSPHORIC ACID IN FLOATS

Additions	Sterilized and Inoculated		Unsterilized and Inoculated		Untreated	
	Acidity c.c.	Citrate-Soluble P_2O_5 mg.	Acidity c.c.	Citrate-Soluble P_2O_5 mg.	Acidity c.c.	Citrate-Soluble P_2O_5 mg.
AT END OF 7 WEEKS						
None	20.00	65.10	10.00	73.37	10.50	70.86
5 gm. Sulfur	188.00	83.88	818.00	97.66	402.00	90.14
15 gm. Floats	31.50	121.44	10.00	159.00	10.75	164.01
5 gm. Sulfur, 15 gm. Floats	535.00	391.88	562.00	434.44	484.00	260.42
AT END OF 14 WEEKS						
None	16.00	68.30	12.10	70.40	11.05	71.00
5 gm. Sulfur	408.00	78.90	1080.00	82.10	648.00	87.50
15 gm. Floats	16.50	123.90	12.10	140.10	11.10	139.60
5 gm. Sulfur, 15 gm. Floats	496.00	491.88	612.00	634.37	458.00	339.49

SUMMARY

The theory recently advanced by the senior author that the oxidation of sulfur by microörgonisms is of value in making available the phosphorus of mineral phosphates has been substantiated by the following results (subject to the limitations of the experiments).

1. One hundred-gm. portions of sea sand, Sassafras loam and a greenhouse soil to which had been added manure, were composted with sulfur and floats and analysed periodically for acidity and available phosphoric acid by the official method. In the sand cultures the acidity of the sulfur-floats mixture increased with the period of incubation until at the end of the eighteenth week it was four times as great as where sulfur

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had been applied alone. That the sulfofication process gained in intensity most markedly after the eighteenth week is supported by the data showing that the available phosphoric acid in the sulfur-floats mixture increased markedly compared with the sulfur treatment from the eighteenth to thirtieth week.

2. The greenhouse soil, being rich in nitrogen and mineral plant-food as well as organic matter proved superior to sand as a culture medium. The increase in acidity was much greater than in sand. The sulfur alone yielded a maximum accumulation larger than that of the sulfur-floats mixture. But since 85 per cent of the phosphoric acid present in the latter was made available it is evident that the acid which resulted from the oxidation of the sulfur in the sulfur-floats mixture had reacted with the tricalcic phosphate. The results obtained with the Sassafras loam differed in degree rather than in kind from those already recorded for the sand and greenhouse soils, showing that the biological changes throughout the experiment were limited in a definite way by the physical and chemical properties of the medium.

3 Analyses for the accumulation of water-soluble phosphoric acid showed increases in all of the mixtures which had received additions of both sulfur and floats.

4. In both soils and sand, the oxidation of sulfur as measured in terms of sulfates, paralleled the increase of available phosphoric acid throughout the incubation period of 30 weeks. The tricalcium phosphate hastened the oxidation of sulfur by furnishing phosphorus for the life activities of the sulfur organisms and by neutralizing the free acid which being formed would tend to inhibit the proper development of the sulfur-oxidizing organisms.

5. Investigation of moisture as a factor in sulfur oxidation revealed the fact that high or low moisture contents retarded the production of acidity, as well as of the citrate and water-soluble phosphoric acid. One and one-fourth optimum moisture conditions appeared to be best in sassafras soil for maximum results in rendering available raw rock phosphate. It was shown also that in a comparison of (1) untreated, (2) sterilized and inoculated, (3) unsterilized and inoculated soils, the biological factor proved influential in the oxidation of sulfur and the making available of raw rock phosphate.

6. Data are advanced which indicate that in composting under farm conditions, one part of sulfur and two parts of floats may constitute a satisfactory combination for the production of available phosphoric acid. While several points are still under investigation, there is good reason to believe that in field practice, inert phosphates may best be made available by sulfur oxidation in a relatively concentrated medium *e. g.* in a compost heap containing a relatively large proportion of both phosphates and sulfur.

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Literature Cited

(1) Aikman, C. M.
1894. Manures and Manuring, p. 84. London.

(2) Ames, J. W., and Boltz, G. E.
1916. Sulfur in relation to soils and crops. Ohio Agr. Exp. Sta. Bul. 292, p. 221-256.

(3) Bassalik, K.
1913. Ueber Silikatzersetzung durch Bodenbakterien und Hefen. *In* Ztschr. Garungsphysiol., Bd. 3, Heft 1, p. 15-42. *Abs. in* Exp. Sta. Rec., v. 31, p. 121.

(4) Boiret, H., and Paturel, G.
1892. The use of sulfate of iron in agriculture. *In* Ann. Agron., v. 18, p. 417-440. *Abs. in* Exp. Sta. Rec., 1893, v. 4, p. 435-436.

(5) Boullanger, E.
1912. L'Action du soufre en fleur sur la vegetation. *In* Compt. Rend. Acad. Sci. (Paris), t. 154, no. 6, p. 369-370.

(6) Bretschneider, P.
1870. Ueber die Löshlichkeit einger Phosphorsäure Verbindungen in reinem und in kolensäure enthaltendem Wasser. *In* Ber. Vers. Stat. Ida-Marienhutte, No. 14, Abs. *in* Jahrb. Agr. Chem., Bd. 13-15, p. 211-213.

(7) Brioux, Ch., and Guerbet, M.
1913. E'volution du soufre dans le sol, étude sur son oxydation. *In* Compt. Rend. Acad. Sci. (Paris), t. 156, no. 19, p. 1476-1479.

(8) Brown, C. W.
1907. The influence of the composition of the medium upon the solvent action of certain soil bacteria. *In* Rpt. Mich. Acad. Sci., no. 9, p. 160-162. *Abs. in* Exp. Sta., v. 20, p. 120-121.

(9) Brown, P E., and Kellogg, E. H.
1914. Sulfofication in soils. Iowa Agr. Exp. Sta. Research Bul. 18, p. 49-111.

(10) Cazeneuve, P., and Nicolle, A.
1892. Ueber die Reaktion des Eisensulfats gegenüber den in der Landwirtschaft angewandten Phosphates. *In* Chem. Centbl., Jahrg. 63, Bd. 2, p. 121.

(11) Degrully, L.
1911. L'Action fertilisante du soufre. *In* Prog. Agr. et Vit., t. 57, p. 321-324.

(12) Demolon, A.
1912. Sur l'action fertilisante du soufre. *In* Compt. Rend. Acad. Sci. (Paris), t. 154. no. 8, p. 524-526.

(13) Demolon, A.
1913. Recherches sur l'action fertilisante du soufre. *In* Compt. Rend. Acad. Sci. (Paris), t. 156, no. 9, p. 725-728.

(14) Dietrich, Th., König, J. and Kiesow, J.
1873. Löslichkeit von Phosphaten in Humuslösung und die Verwendung von rohen Phosphaten. *In* Landw. Ztg. Westfalen u. Lippe, Bd. 29, No. 49, p. 383. *Abs. in* Centbl. Agr. Chem., 1873, Bd. 3, p. 77-79.

(15) Falkner, F.
1844. The Farmer's Treasure, p. 42. New York.

Digitized by Google

(16) FLEISCHER, M.
1883. Die Verwendung schwerlöslichen Phosphate auf mineralischen Bodenarten. *In* Landw. Jahrb., Bd. 12, p. 186-192.

(17) FRAPS, G. S.
1910. Active phosphoric acid and its relation to the needs of the soil for phosphoric acid in pot experiments. Texas Agr. Exp. Sta. Bul. 126, p. 1-72.

(18) GALTSEV, P. E. and YAKUSHKIN, I. V.
1913. Siernyĭ kolchedan i sul'faty zhelieza v ikh vli'ianii na fosforit. *In* Moskov. Sel'sk Khoz, Inst. God. 19, p. 223-232.

(19) GAYLORD, WILLIS
1843. On the preparation and use of manures. *In* Trans. N. Y. State Agr. Soc., v. 2, p. 79.

(20) HALL, A. D.
1910. Fertilizers and Manures. New York.

(21) HARTWELL, B. L., and PEMBER, F. R.
1912. The effect of cow-dung on the availability of rock phosphate. R. I. Agr. Exp. Sta. Bul. 151, p. 165-174, 1 pl.

(22) HEIDEN, E., and HOSAEUS, A.
1873. Ueber die Zersetzbarkeit des Lahn-phosphorites etc. *In* Amts-bl. Landw. Ver. Konigr. Sachsen, No. 5, p. 61; and *in* Landw. Jahrb., Bd. 2, Heft 2, p. 309.

(23) HOLDEFLEISS, F.
1874. Versuch über die Aufschliessung des Phosphorites durch Kompostierung. *In* Ztsch. Landw. Centralver, Prov. Sachsen, Bd. 31, No. 8, p. 197. Abs. *in* Centbl. Agr. Chem., Bd. 7, p. 166-173.

(24) HOPKINS, C. G.
1910. Soil Fertility and Permanent Agriculture. New York. 653 p.

(25) HOPKINS, C. G., and WHITING, A. L.
1916. Soil bacteria and phosphates. Ill. Agr. Exp. Sta. Bul. 190, p. 395-406.

(26) KAPPEN, H., and QUENSELL, E.
1915. Ueber die Umwandlungen von Schwefel und Schwefelverbindungen im Ackerboden, etc. *In* Landw. Vers. Stat., Bd. 86, p. 1-34.

(27) KARMRODT, K.
1872. Ueber die Löslichkeit der Phosphorsäure im Phosphorit und in einigen Dungemitteln. *In* Neue Landw. Ztg., Bd. 21, p. 30, Abs. *in* Centbl. Agr. Chem., Bd. 2, p. 10-11.

(28) KISSLING, R.
1883. Die Verwendung schwerlöslichen Phosphate auf mineralischen Bodenarten. *In* Landw. Jahrb., Bd. 12, p. 193-202.

(29) KOCH, M. A., and KRÖBER, ED.
1906. Einfluss der boden Bakterien verschiedenen Phosphaten. *In* Engrais, t. 21, p. 349.

(30) KOSSOVICH, P. S.
1904. O vzaimodieistvii pitatel'nykh soleĭ v protesessie vospriniatiia rasteniiami mineral'noi pischi. *In* Zhur. Opuitn. Agron. (Russ. Jour. Exp. Landw.), Bd. 5, p. 581-612.

(31) LEIBIG, J.
1840. Chemistry in its Application to Agriculture and Physiology. 1st Edition.

Digitized by Google

(32) LEIBIG, J.
1859. Modern Agriculture, p. 96.

(33) LINT, H. C.
1914. The influence of sulfur on soil acidity. *In* Jour. Indus. Engin. Chem., v. 6, p. 747.

(34) LIPMAN, J. G., McLEAN, H. C., and LINT, H. C.
1916. The oxidation of sulfur in soils as a means of increasing the availability of mineral phophates. *In* Soil Sci., v. 1, no. 6, p. 533-539.

(35) LUPTON, N. T.
1893. The effect of decomposing organic matter on natural phosphate. Ala. Agr. Exp. Sta. Bul. 48, p. 1-10.

(36) McDOWELL, M. S.
1907. Is the phosphoric acid of floats made soluble by rotting manure? *In* Penn. Agr. Exp. Sta. Rpt. 1907-1908, p. 175-178.

(37) MITSCHERLICH, E. A., and SIMMERMACHER, W.
1913. Einige Untersuchungen über den Einfluss des Ammonsulfates auf die Phosphatdüngung bei Haferkulturen. *In* Land. Vers. Stat., Bd. 79-80, p. 71-96.

(38) MOSES, O. A.
1882. Mineral resources of the United States for 1882. *In* U. S. Geol. Survey Rpt. 1883, p. 512.

(39) NEDOKUCHAEV, N.
1913. O vlianii norvezhskoĭ selitry i siernokislago ammiake na usvoenie fosfornoĭ kisloty fosforita. *In* Zhur. Opuitn. Agron. (Russ. Jour. Exp. Landw.), Bd. 14, p. 1-21.

(40) OHIO AGR. EXP. STA.
1907. The maintenance of fertility. Ohio Agr. Exp. Sta. Bul. 183, p. 195-225.

(41) PFEIFFER, TH., and THURMANN, H.
1896. Ueber das Verhalten einiger Phosphate bei der Kompostierung. *In* Landw. Vers. Stat., Bd. 47, Heft 4/5, p. 343-356. *Abs. in* Jahrb. Agr. Chem., Bd. 19, p. 133-136.

(42) PRIANISHNIKOV, D.
1905. Ueber den Einfluss von Ammoniumsalzen auf die Aufnahme von Phosphorsäure bei höherer Pflanzen. *In* Ber. Deut. Bot. Gesell., Bd. 23, No. 1, p. 8-17; also see Ucheniye ob Udobrenii, 4th ed., 1912, p. 239. Moscow.

(43) PRIANISHNIKOV, D.
1913. Ucheniye ob Udobrenii, 4th ed., 1912. Moscow.

(44) RUSSELL, E. J.
1915. Soil Conditions and Plant Growth. 3rd Edition, London.

(45) SACKETT, W. G., PATTEN, A. J., and BROWN, C. W.
1908. The solvent action of soil bacteria upon the insoluble phosphates of raw bone meal and natural raw rock phosphate. Mich. Agr. Exp. Sta. Spec. Bul. 43, p. 1-30.

(46) SAUSSURE, THEODORE DE.
1884. Recherches chimiques sur la Vegetation, p. 261. Paris.

(47) SCHULOV, Iv.
1900. Rastvoriaiuschee dieĭstv'ie ammiachnykh soleĭ na fosforit. *In* Izv. Moskov. Selsk. Inst. (Ann. Inst. Agron. Moscow), God 8 (1912) Kniga 2, p. 161-167.

Digitized by Google

(48) SHUTT, F. J.
1897. Report of the chemist: The fermenting of manure with finely ground mineral phosphate. *In* Canada Exp. Farms Rpts. 1896, p. 196.
(49) SHEDD, C. M.
1914. The relation of sulfur to soil fertility. *In* Ky. Agr. Exp. Sta. Bul. 188, p. 595-630.
(50) SÖDERBAUM, H. G.
1905. Zur Kenntnis der Factoren, welche die Düngewirkung der Knochenmehl Phosphorsäure beeinflussen. *In* Landw. Vers. Stat., Bd. 63, No. 3-4, p. 247-262.
(51) STOKLASA, JUL., DUCHACEK, F., and PITRA, J.
1900. Ueber den Einfluss der Bakterien auf die Knochenzersetzung. *In* Centbl. Bakt. (etc.), Abt. 2, Bd. 6, p. 526-535.
(52) TOTTINGHAM, W. E., and HOFFMANN, C.
1913. Nature of changes in the solubility and availability of phosphorus in fermenting mixtures. Wis. Agr. Exp. Sta. Research Bul. 29, p. 273-321.
(53) TRUOG, E.
1912. Factors influencing the availability of rock phosphate. Wis. Agr. Exp. Sta. Research Bul. 20, p. 17-51.
(54) VERMOREL, V., and DANTONY, E.
1913. Contribution à l'etude du soufre et des pyrites de fer employés comme engrais. *In* Engrais, t. 28, p. 1304-1306.
(55) WARINGTON, R.
1900. The comparative value of nitrate of sodium and sulfate of ammonium as manures. *In* Jour. Roy. Agr. Soc. England, p. 306-346.
(56) WHEELER, H. J.
1913. Manures and Fertilizers, xxi+389 p., 1 pl., 62 fig., New York.

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THE ORGANIC MATTER OF THE SOIL: II. A STUDY OF CARBON AND NITROGEN IN SEVENTEEN SUCCESSIVE EXTRACTS; WITH SOME OBSERVATIONS ON THE NATURE OF THE BLACK PIGMENT OF THE SOIL[1]

By

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Introduction

It has already been shown in the first paper of this series (2) that:

1. Humus, the "matière noire" of Grandeau (3), does not consist entirely of a black compound or compounds but contains a large proportion of almost colorless substances, the presence of which is normally masked by the black color.

2. Extracts can be obtained from unchanged vegetable materials which are very similar in solubility, color, etc., to "humus" extracts prepared from soils.

3. A 4 per cent solution of sodium hydroxide does not extract either the same substances or the same quantity of substances as does a 4 per cent solution of ammonium hydroxide.

4. All of the mineral soils tested contain a "soil pigment" which is soluble in ammonia and very dilute sodium hydroxide to form intensely black solutions, but which is insoluble in sodium hydroxide of 4 per cent concentration.

5. This soil pigment contains a relatively small proportion of the soil nitrogen and it was suggested that it probably had but little importance, *per se*, in the problem of soil fertility.

In view of the above findings it seemed advisable to attempt to isolate this soil pigment in a pure form. Such an attempt is recorded in this paper. It is to be regretted that the final preparations contain such a high percentage of ash as probably to invalidate the ultimate analyses, but it is impossible to carry the investigation further at the present time, and the results to date seem worthy of record.

[1] The work reported in this paper was carried out while the author, now Associate Agricultural Bio-Chemist, was Associate Soils Chemist.

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Experimental

The soil selected for this investigation was from a bulk sample of the first 6 inches of the Fargo silt loam type (4), the air-dry soil containing 12.70 per cent of moisture. The soil contained 9.90 per cent of carbonate CO_2, 10.04 per cent of organic carbon and 0.908 per cent of nitrogen, all calculated on the basis of oven-dry soil.

In the above mentioned paper (2) the writer has shown that while the soil pigment is insoluble in 4 per cent sodium hydroxide, it is readily soluble in the dilute alkaline solution formed when distilled water is added to the soil residue remaining after siphoning off the 4 per cent sodium hydroxide solution. An attempt was made to utilize this phenomenon for the purpose of separating the soil pigment. One kg. of the air-dry soil, ground to pass a 1-mm. sieve, was leached in 50 gm. lots on filters with 1 per cent HCl until the filtrate gave no test for calcium, and the excess of acid removed by washing with water. The leached soil was then removed to 2½-liter bottles, the soil residue from 100 gm. of the original air-dry soil being placed in each bottle and 2 liters of 4 per cent NaOH added to each. The bottles were shaken several times each day for 3 days and then the soil was allowed to settle until the supernatant liquid appeared to be free from clay, 3 to 4 days usually being allowed. The supernatant liquid was siphoned off as completely as possible without disturbing the soil, measured and united in a large container, 16,900 c.c. being removed. This constituted "NaOH Solution A." Fresh 4 per cent NaOH solution was added to the soil residue until 2 liters of NaOH were again contained in each bottle and the process repeated until there had been 9 successive extractions with 4 per cent NaOH.

Carbon and nitrogen determinations were made of each solution, the carbon being estimated by wet combustion in the apparatus figured in the preceding paper (2), corrections being made for the CO_2 of carbonates in the sodium hydroxide solutions.[1]

The color of the solutions rapidly decreased in intensity, the last two extractions being practically colorless.

Following the nine extractions with sodium hydroxide the contents of one of the bottles was made slightly acid with sulfuric acid, the precipitated soil washed by decantation until practically free from sulfates, then poured upon a filter and washed free of sulfates, removed from the filter, dried, weighed and powdered to pass a 0.5-mm. sieve. From the

[1] It was impossible to secure accurate organic carbons on the large volume (45,350 c.c.) of HCl extract and washings, so that the organic carbon in this portion was determined by difference.

Ten gm. of the air-dry soil were leached on an alundum filter with 1 per cent HCl until free from calcium, and washed free of chlorides with distilled water. The soil was then washed from the filter into an evaporator, dried and weighed. The 10 gm. of original air-dry soil, containing 0.8764 gm. of organic carbon, gave 6.4452 gm. of dry leached soil containing (1) 13.12 per cent, and (2) 13.10 per cent, or an average of 13.11 per cent, equivalent to 0.8450 gm. of organic carbon. The HCl leachings therefore dissolved 0.0314 gm. of organic carbon.

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original 87.30 gm. of soil (dry) there was obtained a residue weighing 41.5 gm. or 47.53 per cent of the original soil weight. This residual soil contained 6.27 per cent of organic carbon and 0.346 per cent of nitrogen.

A "humus" determination was made on this residual soil by shaking 5 gm. of the sample with 750 c.c. of 4 per cent NH_4OH for one week, flocculating the clay with ammonium carbonate, filtering and evaporating 250-c.c. portions to dryness in tared silica dishes. Duplicate determinations gave 8.38 and 8.45 per cent of the residual soil as ash-free humus of an average of 8.42 per cent. The humus-ash percentages were 1.76 and 1.80 per cent.

The "humus" solution was jet black and on drying the product scaled away from the dish in flakes where the deposit had any appreciable thickness. The dissolved material apparently consisted of the black soil pigment combined with ammonia, inasmuch as the product after drying at 105° C. for 12 hours was still readily soluble in water. Detmer (1) has observed that a soluble "ammonium humate" can be formed by dissolving "humic acid" in ammonia and evaporating the mixture to dryness. The properties of his "salt" resemble very closely those of the writer's product.

To the remaining nine soil residues, distilled water was added to 2 liters volume, the mixture thoroughly shaken and 5 gm. of crystallized sodium sulfate added to each bottle to flocculate the clay. After several shakings the soil was allowed to settle and the black supernatant liquid siphoned off, this constituting Pigment Solution 1. Additional 0.25 per cent sodium sulfate solution was added to the residue and the process repeated until six pigment solutions had been removed.[1]

Pigment Preparation I was obtained from the combined Pigment Solutions 1, 2 and 3, and the remaining solutions 4, 5 and 6 furnished Pigment Preparation II (see below).

The residual soil from the pigment extractions was acidified slightly with sulfuric acid, washed by decantation to the absence of acid reaction, poured upon filters and washed to the absence of sulfates, dried, weighed and powdered to pass a 0.5-mm. sieve. There was thus obtained a light gray residue weighing 330.5 gm. as compared with an original weight of 785.7 gm. (900 gm. air-dry) and containing 3.59 per cent of organic carbon and 0.211 per cent of nitrogen.

Inasmuch as it was thought probable that there was still some of the "soil pigment" remaining in the residue, "humus" was determined on the resulting residue. Two 15-gm. portions were leached with 1 per cent HCl and washed free of chlorides as in the usual humus method. This treatment was resorted to in order to get the soil in as near as possible the same physical state as it would have been had it not been previously dried and ground. The 15 gm. of soil were then added to 750-c.c. portions

[1] Fifteen c.c. of 20 per cent NaOH were added to each 2000 c.c. of solution used for the extraction of Pigment Solutions 5 and 6.

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of 4 per cent NH_4OH in separate bottles and shaken for one week in a shaking machine so arranged that the bottles were rotated sidewise "wheel fashion" so that the soil was continually falling through the solution. At the end of a week the bottles were removed, the clay precipitated in a portion of the solution with K_2SO_4 and carbon determined on the jet black solution as in the writer's earlier directions (2). The remaining portion was used for "humus" determinations after flocculating the clay with ammonium carbonate as in Rather's (5) method. The residual soil was found to contain 1.54 per cent of humus (ash-free), 0.74 per cent of "humus ash," and 0.95 per cent of "humus carbon" indicating 62.25 per cent of carbon in the humus. This carbon percentage agrees fairly well with the carbon content of Pigment Preparation I, *i. e.*, 61.33 per cent (see later).

The summarized analytical data are shown in Tables I and II. Figure 1 is a graphic representation of the data of Table II.

The analytical results obtained on this series are conclusive proof of the accuracy of my wet combustion apparatus for the determination of organic carbon. The increase of 1.534 gm. of carbon amounts to an error of 1.76 per cent of the total carbon. Inasmuch as carbon was determined on 50-c.c. portions of the solution, there would have been enough solution to make 5,012 carbon determinations, not considering the 45,350 c.c. of the HCl extract; a gain of 1.534 gm. of carbon, therefore, amounts to an average gain of only 0.0003 gm. of carbon per determination. A nitrgoen gain of 0.1590 gm. N is similarly an average gain of only 0.00013 gm. N per determination, not counting errors in the nitrogen content of the original soil nor of the final soil residue.

Pigment Preparation 1. Pigment Solutions 1, 2 and 3 were united and acidified with sulfuric acid. A jet black flocculent precipitate separated leaving the supernatant liquid absolutely colorless. The precipitate was washed by decantation with distilled water until the supernatant liquid began to take on a brown tint showing that the pigment was beginning to form a colloidal solution. Sufficient sodium hydroxide solution was then added to form a 0.2 per cent solution. The precipitated pigment was dissolved to a jet black solution, which was only faintly alkaline to litmus. This solution was poured upon a large filter and the filtrate diluted to 7 liters in volume. One liter of sodium hydroxide solution containing 320 gm. of sodium hydroxide was then added, thus making the solution to contain 4 per cent of sodium hydroxide. The black pigment was precipitated in this concentration of alkali, leaving a supernatant liquid which appeared very dark when in large bulk but which was relatively light colored when in small volume. This supernatant liquid was analyzed for organic carbon and nitrogen and gave a ratio of C/N of 15.77. This is decidedly lower than the C/N ratio of the original solution, 21.0, and would indicate that the substances dissolved here were perhaps normally soluble in 4 per cent NaOH. It is

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to be noted that this ratio of 15.77 logically follows the C/N ratio of NaOH solution 1.

The supernatant liquid was removed from the pigment as completely as possible by centrifuging, and the solid mass of pigment added to 8 liters of distilled water in which it readily dissolved to form a jet black solution. This solution was filtered by gravity through double filters and

TABLE I

SUMMARIZED ANALYTICAL DATA ON THE SOILS AND SOLUTIONS[1]

	Volume of Solution Removed from 1 kg. Soil[2]	Organic Carbon			Nitrogen		
		Per cent I	Per cent II	Per cent Av.	Per cent I	Per cent II	Per cent Av.
	c.c.						
1% HCl Extract	45,350	0.353	0.368	0.360	0.025	0.023	0.024
NaOH Solution—							
A	16,900	3.353	3.323	3.338	0.363	0.364	0.363
B	17,100	1.126	1.145	1.136	0.138	0.138	0.138
C	17,300	0.640	0.626	0.633	0.080	0.080	0.080
D	17,600	0.442	0.424	0.433	0.053	0.053	0.053
E	17,700	0.298	0.299	0.298	0.033	0.033	0.033
F	17,300	0.252	0.252	0.252	0.026	0.027	0.026
G	17,500	0.173	0.180	0.177	0.018	0.017	0.018
H	17,000	0.143	0.151	0.147	0.012	0.012	0.012
I	15,900	0.101	0.108	0.104	0.010	0.009	0.009
Residual Soil from NaOH Solution I	.....	3.007	2.953	[3]2.980	0.163	0.165	[3]0.164
Pigment Solution—							
1	15,890	0.168	0.160	0.164	0.008	0.009	0.009
2	18,890	0.475	0.474	0.475	0.020	0.021	0.020
3	17,112	0.595	0.591	0.593	0.029	0.029	0.029
4	14,445	0.292	0.290	0.291	0.015	0.015	0.015
5	14,110	0.164	0.183	0.173	0.006	0.006	0.006
6	15,890	0.104	0.102	0.103	0.006	0.003	0.004
Residual Soil from Pigment Solution 6	.....	1.521	1.503	1.512	0.088	0.089	0.088
Total	.....	.....	.....	10.189	.....	.....	0.927
Original Soil	.....	10.070	10.020	10.040	0.904	0.913	0.908

[1] All percentages are based on the weight of the original soil (dry at 105°).

[2] The volume for the pigment solutions has been increased 1/9 to correspond to 1 kg. of soil instead of 900 gm. actually extracted (see text).

[3] Not included in the additions for "Total."

the filtrate acidified slightly with sulfuric acid. The precipitate was washed by decantation until it began to form a colloidal solution, then poured on a filter and washed until practically free from sulfates. The large mass of gelatinous precipitate, probably approximating 800 c.c. in bulk, was dried on a water bath. In drying it cracked into small pieces which resembled anthracite in color and hardness when perfectly dry. The yield was approximately 16 gm.

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The crude pigment was powdered, extracted on an alundum filter with hot water to remove traces of sulfates, then extracted in a Soxhlet apparatus with alcohol. The extract rapidly darkened and finally became deep orange in color. On evaporating off some of the alcohol and cooling, a very considerable quantity of colorless crystals separated. These crystals have not as yet been identified.

The extracted pigment was dried at 105° C. and analyzed for carbon, hydrogen, nitrogen and ash in the usual manner. The product as prepared forms a jet black amorphous powder, insoluble in alcohol, ether, chloroform or any of the usual organic solvents, insoluble in dilute acids,

TABLE II

SHOWING THE PERCENTAGE OF THE TOTAL CARBON AND NITROGEN IN THE DIFFERENT SOILS AND SOIL EXTRACTS

	Gm. C.	Per cent Total C.	Gm. N.	Per cent Total N.	C/N Ratio
1000 gm. Original Soil	87.640	.....	7.9300	.....	11.05
1% HCl Extract	3.137	3.58	0.2192	2.76	14.32
NaOH Solution—					
A.....	29.139	33.25	3.1730	40.01	9.18
B.....	9.910	11.31	1.2034	15.17	8.24
C.....	5.524	6.30	0.6972	8.79	7.92
D.....	3.782	4.32	0.4611	5.81	8.20
E.....	2.607	2.98	0.2867	3.61	9.09
F.....	2.199	2.51	0.2318	2.92	9.49
G.....	1.756	2.00	0.1505	1.90	11.67
H.....	1.278	1.46	0.1054	1.33	12.13
I.....	0.919	1.05	0.0668	0.84	13.76
Residual Soil from NaOH Solu. I	[1]26.020	[1]29.69	[1]1.4350	[1]18.09	18.12
Pigment Solution—					
1.....	1.421	1.62	0.0747	0.94	19.03
2.....	4.152	4.74	0.1813	2.29	22.90
3.....	5.181	5.91	0.2498	3.15	21.06
4.....	2.537	2.89	0.1271	1.60	19.96
5.....	1.531	1.75	0.0522	0.66	29.34
6.....	0.901	1.03	0.0365	0.29	24.66
Residual Soil from Pig't Solu. 6	13.200	15.06	0.7730	9.75	17.08
Total	89.174	101.76	8.0900	101.82	.....

[1] Not included in the additions for "Total."

soluble in concentrated sulfuric acid to a black solution, dissolving slightly in hot concentrated nitric acid to a red-brown color, soluble in ammonia or very dilute solutions of the fixed alkalis to intensely black solutions, from which the pigment can be again precipitated by solutions of the heavy metals or by acidification.

When alkaline solutions of the pigment are dialyzed in collodion bags, the excess of alkali escapes but no pigment passes through. In this manner neutral solutions of the pigment can be easily prepared, which yield a black water-soluble residue when evaporated to dryness. These probably correspond in nature to the ammonium compound obtained by evaporating an ammoniacal solution of the pigment. Whether we are

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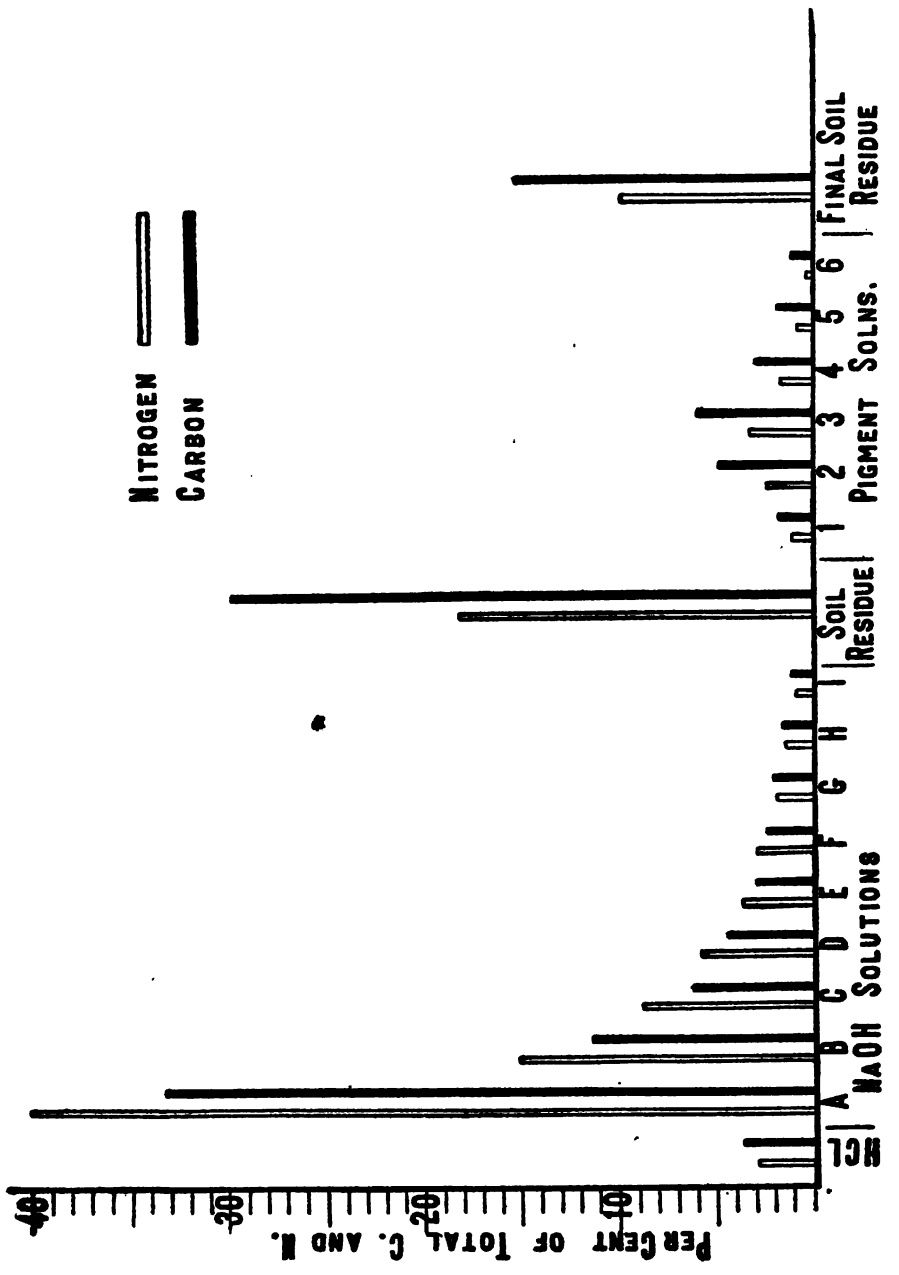

Fig. 1.—A graphic representation of the data in Table II.

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dealing with a true salt in this instance can be decided only by further experimentation on purer material. The following analytical data were obtained on the sample:

Ash determinations indicated 37.50, 37.74 and 37.16 per cent or an average of 37.47 per cent of ash.

Organic carbon (by CuO in oxygen) determinations gave 61.28, 61.59 and 61.12 per cent C, or an average of 61.33 per cent of carbon calculated to an ash-free basis.

Hydrogen determinations gave 4.35, 4.25 and 4.37 per cent hydrogen, or an average of 4.36 per cent calculated ash-free.

Nitrogen determinations gave 2.79 and 2.82 per cent N, or an average of 2.81 per cent calculated ash-free.

Sulfur was not tested for because of the high ash content and also because sulfuric acid had been used to precipitate the pigment.

These analyses therefore would indicate an approximate content of:

Carbon	61.3 per cent
Hydrogen	4.3 per cent
Nitrogen	2.8 per cent
Oxygen	31.6 per cent

It is useless to compare these analyses with the analyses of "humic acids," etc. in the literature until this product can be prepared in a purer state. It is of interest to note, however, that the analyses approximate those of Detmer's (1) "humic acid," which contained 60.74 per cent C, 4.48 per cent H, and 35.78 per cent O. Detmer's product contained smaller amounts of nitrogen, ranging from 0.79 per cent to 0.18 per cent, and he was inclined to the belief that his "humic acid" was nitrogen-free. The physical properties of his material are almost identical with those of the soil pigment used by the writer. He does not state, however, whether or not his "humic acid" is insoluble in 4 per cent NaOH and soluble in the very dilute solutions; this would appear to be the distinguishing feature of the soil pigment.

The high ash content consists presumably of colloidal clay which was not removed by the preliminary treatment. This clay undoubtedly holds a certain amount of combined moisture that was not driven off by the drying at 105° C. so that it appears probable that the hydrogen content as determined is too high. Some diminution of ash could be secured by dissolving the pigment in ammonia and precipitating the clay with ammonium carbonate. This was what was actually done in the humus determination on the residual soil from NaOH solution 1. This soil residue was found to contain 8.42 per cent of humus with 1.78 per cent of humus ash, or an ash content of only 17.45 per cent in the materials not precipitated by the ammonium carbonate. The ammonia treatment would be far more rapid than the sodium hydroxide process but was not

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utilized because of the danger of introducing ammonia into the compound and thus increasing the nitrogen content. Inasmuch as the writer's analyses now show the approximate nitrogen content, it is extremely desirable that some one should attempt to isolate the pigment by the ammonia process. Grandeau (3) found that when an ammoniacal solution of humus containing 53 per cent of ash was dialyzed against distilled water for 36 hours, the resulting residue in the dialyzer had an ash content of only 8 per cent. Perhaps this method could be employed in freeing the soil pigment from ash.

Pigment Preparation 2. This sample of pigment, weighing approximately 7 gm. was prepared from Pigment Solutions 4, 5 and 6, following much the procedure as was used for Pigment Preparation 1, with the exceptions that the solution of the pigment in dilute alkali was not precipitated by adding NaOH up to 4 per cent concentration and that hydrochloric acid was used for acidification instead of sulfuric. The final extractions were the same for both preparations. The following analytical data were obtained on the sample:

Ash determinations indicated 50.77 and 51.57 per cent, or an average of 51.17 per cent of ash.

Organic carbon (by CuO in oxygen) determinations gave 55.82 and 56.23 per cent C, or 56.03 per cent of organic carbon calculated to an ash-free basis.

Hydrogen determinations gave 5.89 and 6.36 per cent, or 6.13 per cent calculated ash-free.

Nitrogen determinations gave 2.94 and 3.02 per cent N, or an average of 2.98 per cent calculated to an ash-free basis.

These analyses would indicate an approximate content of

Carbon	56.03 per cent
Hydrogen	6.13 per cent
Nitrogen	2.98 per cent
Oxygen	34.86 per cent

It will be noted that this preparation contains appreciably more hydrogen and less carbon than Preparation 1. This is exactly what would be expected from a sample having a higher ash content, if the ash consisted of clay. Apparently the clay has retained enough water of constitution to vitiate entirely the hydrogen analysis and to lower the carbon precentage appreciably.

Summary

A sample of soil of the Fargo silt loam type was leached with 1 per cent hydrochloric acid and then extracted 9 consecutive times with fresh portions of a 4 per cent sodium hydroxide solution. A sample of the residual soil was removed at this point and analyzed, while the remain-

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ing portions were further extracted for 6 consecutive times with very dilute (approximately 0.15 per cent concentration) sodium hydroxide solution. Following these extractions the remaining soil residue was dried and analyzed. The following conclusions are evident from the analytical data:

1. The writer's earlier observation that 4 per cent sodium hydroxide solution does not dissolve the black soil pigment is confirmed.

2. The soil pigment is soluble in very dilute sodium hydroxide solutions but precipitates from solution on the addition of sodium hydroxide in sufficient amount to make a 4 per cent solution.

3. The soil pigment is also precipitated from solution by salts of the heavy metals and by acidification; is not dialyzable and forms a stable water-soluble compound with ammonia when an ammoniacal solution is evaporated to dryness.

4. Two attempts were made to prepare the soil pigment in pure form but the resulting products contained such a high content of ash (37.47 per cent and 51.17 per cent, respectively) as probably to render the ultimate analysis unreliable. The analysis of the product with the least ash content gave C., 61.3 per cent; H., 4.3 per cent; N., 2.8 per cent; O., 31.6 per cent; all calculated to an ash-free basis.

5. The first six extractions with NaOH removed relatively more nitrogen than carbon from the soil, but the remaining three NaOH extractions as well as the six pigment solutions contained relatively more carbon than nitrogen. The final soil residue had a ratio of C/N very much higher than that of the original soil.

Literature Cited

(1) Detmer, W.
1871. Die natürlichen Humuskörper des Bodens und ihre landwirthschaftliche Bedeutung. *In* Landw. Vers. Stat., Bd. 14, p. 248. Reprinted *in* Jahresber. Agr. Chem., 1870-72, p. 68-72.

(2) Gortner, R. A.
1916. The organic matter of the soil: I. Some data on humus, humus-carbon and humus-nitrogen. *In* Soil Sci., v. 2, p. 395-442.

(3) Grandeau, Louis.
1872. Recherches sur le rôle des matières organiques du sol dans les phénomènes de la nutrition des végétaux. *In* Compt. Rend. Acad. Sci. [Paris], t. 74, p. 988-991.

(4) Marbut, C. F., Bennett, H. H., Lapham, J. E., and Lapham, M. H.
1913. Soils of the United States. U. S. Dept. Agr. Bur. Soils Bul. 96. 791 p., 13 fig., 2 maps.

(5) Rather, J. B.
1911. An improved method for the estimation of humus. *In* Jour. Indus. Engin. Chem., v. 3, p. 660-662.

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FERRIFICATION IN SOILS[1]

By

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Introductory

Iron has long been known as an essential plant-food constituent. The experiences of the early plant physiologists indicated that the formation of chlorophyl was dependent upon the presence of the element iron. It was recognized, of course, that other constituents were likewise necessary, but in the absence of iron plants soon took on a yellowish, or blanched appearance known as chlorosis, and died. Analyses failed to show the presence of iron in chlorophyl and hence its importance was concluded to be due to a stimulative catalytic, or some other unknown action.

Molisch (8), Raulin (11) and Benecke (1) believed that iron was necessary for the formation of protoplasm and that the chlorosis brought about by its absence was a secondary effect.

Many experimentalists have confirmed the work of the early investigators in showing that iron is essential for the proper development of plants, but little light has been thrown on the question of its exact function in the plant cell. Experiments along such lines would certainly prove extremely interesting and valuable.

While, therefore, the importance of iron to plants has long been recognized, it has always been believed that there was sufficient present in the proper condition in all soils to keep plants supplied for an indefinite period. Certain experiments have shown, however, that applications of iron salts to some soils may prove beneficial. Griffith (4) secured large increases in beans when ferrous sulfate was applied to the soil. Increases were likewise secured when the same salt was applied to turnips, meadow hay, mangel-wurzels, potatoes, wheat, tobacco, onions and cabbage. While there is some question regarding the exact action and cause of the value of the ferrous sulfate used in these experiments, Griffith reports the soils used as extremely low in iron content and the assumption is that the addition of iron benefited the crops. Soils, in various localities, have been found deficient in the element iron, and plants grown on them show this deficiency by their typical chlorotic appearance. It seems therefore, that iron fertilization of some soils may be rather important.

On the other hand, Halligan (6) believes that enough iron is soluble in the weak soil acids to supply the needs of plants so that this material

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need not be considered when applying fertilizers. Russell and Hutchinson (13) assume likewise that iron salts are unnecessary as manures. Hall (5) is even more emphatic, in declaring the use of iron sulfate either for farm or garden crops as unnecessary. He insists that no direct evidence has yet been adduced for a beneficial effect of iron salts either on color or yield, and that experiments have never been conducted in a manner to raise the supposed increase due to the iron, beyond the range of experimental error.

While it must be admitted that the experiments along this line are rather unsatisfactory and broad conclusions should not be drawn from them, it seems that the importance of iron is too great and the possibility of its absence, at least of its absence *in the proper form*, is too real, to dismiss the question of iron fertilization with an unqualified statement that it is *never* necessary. Future experiments may demonstrate its value on certain soils, and in any case the value of iron and the importance of insuring its presence in the proper condition should be kept in mind.

Iron occurs in soils in inorganic and organic forms, largely unavailable, and it is necessary therefore that iron oxidation or ferrification as Lipman (7) calls it, occur in soils if plants are to secure the iron they need.

Regardless, therefore, of the great question of the need of iron fertilization, there is much of interest and value attached to the problem of ferrification in soils.

Does this process occur in soils? Is it different in different soils? What conditions govern its occurrence? How may it be controlled? These are some of the questions which immediately arise in a consideration of the problem. Then too, there is the fundamental question of the measurement of the process by laboratory methods. Can accurate methods be devised? If so, how? Broad practical applications of such a problem follow as a matter of course upon the more technical beginnings. Assuming definite, positive results, the whole question of iron fertilization, soil treatment to make iron available, and other problems, would be opened up. In short, the value of experiments on iron oxidation in soils seemed eminently sufficient to warrant considerable attention. No other work along such a line has been undertaken so far as the authors are aware and while the results are far from definite, it is felt that sufficient data were secured to warrant more or less general conclusions and to open up the subject for further study. If nothing else is accomplished by this work, it is clearly shown that chemical and bacterial processes occur so intimately associated in soils that differentiation is often difficult, if not quite impossible. Ferrification, like many other processes occurring in soils, is shown to be partly a chemical process and partly due to a bacterial action. There is some question which is the more

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important but from these results the chemical oxidation of iron appears to be much greater than the bacterial action. Later results on a wider variety of soils may modify this conclusion. No attempt has been made yet to isolate and describe iron-oxidizing soil bacteria. Several organisms have been isolated from water supplies and described by Rullmann (12), Ellis (23), Molisch (9), Schorler (14) and others but it is regarded as extremely doubtful if these organisms occur in soils.

Probably an entirely different group of organisms is responsible for iron oxidation in soils. An interesting study is indicated here. The present work, however, considers only the ferrifying power of soils in general without regard to specific organisms. It is an attempt to answer, in a general way at least, the questions which have been mentioned above.

The first problem in connection with the work was, of course, the devising of chemical methods which would allow of accurate determinations of ferrous or ferric iron in soils. Just as has been the case in the study of other bacterial processes in soils, it was found that the chemical methods which must be employed were entirely unsatisfactory for application to soils. The first section of the work considers briefly, therefore, the tests that were carried out in order to secure a satisfactory means of determining either ferrous or ferric iron in soils. Such a method was absolutely essential to a measurement of ferrification.

Methods for the Determination of Iron in Soils

As the work was originally planned it required a method for the determination of ferrous iron in the presence of ferric, and it seemed advisable to test the methods available.

Many questions arose in this connection. Does ferrous iron occur in the soil? Would the methods available detect all the ferrous iron present? What effect would the strength and kind of acid have upon the results? If the ferrous compounds were all extracted from the soil, would they not be readily oxidized to the ferric form? Does the organic matter of the soil interfere in the determinations? The following preliminary tests were planned to answer some of these questions, if possible.

The colorimetric methods for the determination of total and ferrous iron as described by Schreiner and Failyer (15), Wiley (16), and in the "Standard Methods of Water Analysis," all involve numerous reagents and a difficult procedure. Since several of these series required sixty to seventy determinations, these colorimetric methods were not considered.

The following method for determining the total iron was tested and found to give quite satisfactory results.

Weigh out 5 gm. of soil and transfer to a nickel crucible. Thoroughly

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mix 15 gm. of sodium peroxide with the soil. Heat at a low heat for 45 minutes. Transfer to a 500-c.c. volumetric flask. Add hydrochloric acid until the material in the flask takes on a yellow fluffy appearance. Allow to cool, make up to the mark, and draw off 100 c.c. of the clear solution. Heat to boiling with nitric acid and precipitate the iron with ammonium hydroxide. Boil, filter out the precipitate and wash with hot water. Dissolve the precipitate with sulfuric acid, reduce the iron with zinc and titrate with potassium permanganate.

A modification of this method, which reduced the operations somewhat was tested and found to give equally good results. Draw off 100 c.c. of the clear solution in the above method. Add 20 c.c. of concentrated sulfuric acid and heat to boiling. Reduce with stannous chloride until a colorless solution is obtained. Cool, add 10 c.c. of mercuric chloride and allow to stand for 10 minutes. Add 25 c.c. of manganese sulfate and titrate with potassium permanganate.

The two methods were compared and the results agreed very closely, as shown in Table A.

TABLE A

COMPARISON OF METHODS FOR DETERMINING TOTAL IRON IN SOIL

Sample No.	c.c. $KMnO_4$	Per cent Fe	Method
1	2.75		
2	2.65		Precipitated with NH_4OH
3	2.65		(First Method)
4	2.65	1.999788	
1	2.65		
2	2.60		Not precipitated with NH_4OH
3	2.70		(Second Method)
4	2.65	1.992270	

The latter method being found suitable for the determination of total iron, the next step was to develop a method whereby the ferrous iron could be determined in the presence of the ferric forms.

Morrison and Doyne (10) determined ferrous iron by digesting 10 gm. of soil in a water bath for 2 hours with 100 c.c. of 1.3 sulfuric acid; this was made up to 250 c.c., filtered, and an aliquot titrated with $KMnO_4$. Their results indicate ferrous iron in the soil in amounts as high as 30,000 pounds per acre, which is without question an impossible amount. Their results with additions of peat to the soil, indicate a decrease in the ferrous iron content.

Normal acetic acid was used but abandoned because they state that it merely serves to indicate that the amount of soluble ferrous iron must be very small. They conclude that the existence of ferrous iron in normal soils to any extent seems improbable.

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Method I

In this first series of experiments to determine ferrous iron, 10 gm. of a sandy loam soil were used, to which was added 1 gm. of ferrous sulfate. This mixture was placed in an apparatus as shown in figure 1.

One hundred c.c. of normal acetic acid were placed in the separatory funnel, and after carbon dioxide had passed through the soil for several minutes, the acid was allowed to percolate through the soil. Following this, the soil was washed with cold distilled water until the volume of the filtrate was 500 c.c.

The filtrate was titrated with potassium permanganate and the amount of ferrous iron calculated, with the results shown in Table I.

TABLE I

FERROUS IRON CALCULATED ACCORDING TO METHOD I

.0015036 gm.	of ferrous iron in the soil alone.
.0367800 gm.	of ferrous iron added in the form of ferrous sulfate.
.0382836 gm.	of ferrous iron added + that in the soil.
.0219906 gm.	of ferrous iron found in filtrate after extraction.
.0162930 gm.	of ferrous iron not reclaimed.

Since all precautions were taken to avoid oxidation of the iron after being placed in the apparatus, the amount of ferrous iron unaccounted for must have been due to an oxidation during the process of weighing and transferring to the apparatus, or by using an acid of insufficient strength to extract all the ferrous iron. A further loss might be accounted for by the adsorptive power of the soil.

Method II

In order to determine the effect of acids upon the amounts of iron recovered, the normal acetic acid used in the preceeding experiment was replaced with normal hydrochloric acid. The method was otherwise carried out in the same manner.

TABLE II

FERROUS IRON CALCULATED ACCORDING TO METHOD II

.0367800 gm.	of iron added in the ferrous sulfate.
.0205747 gm.	of iron reclaimed.
.0162053 gm.	of iron not reclaimed.

Again it is seen that probably because of oxidation and adsorption, a loss of ferrous iron occurred.

Method III

The acids in the above two methods have not remained in contact with the soils for any given length of time. Therefore an apparatus was set up as shown in figure 2.

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Fifty-gm. samples of soil were placed in the milk bottles and carbon dioxide run into the bottles until they were thoroughly filled. Acetic acid was then added and the contents of the bottles shaken for varying lengths of time as shown in Table III.

TABLE III

FERROUS IRON CALCULATED ACCORDING TO METHOD III

Sample No.	Amount of Soil and Iron Used	Time Shaken	Solvent Used	Av. gm. Fe Extracted per gm. of Soil	Gm. Iron Added	Gm. Iron Not Reclaimed
1	50 gm. soil.....	10 min.	150 c.c. N. acetic	.00012250	.....	.....
2	50 gm. soil.....	10 min.	150 c.c. N. acetic	.00012050	.....	.....
3	50 gm. soil.....	10 min.	150 c.c. N. acetic	.00012050	.....	.....
4	50 gm. soil.....	10 min.	150 c.c. N. acetic	.00012050	.....	.....
5	50 gm. soil.....	55 min.	200 c.c. N. acetic	.00013215	.....	.....
6	50 gm. soil.....	75 min.	200 c.c. N. acetic	.00012565	.....	.....
7	50 gm. soil.....	90 min.	200 c.c. N. acetic	.00013215	.....	.....
8	50 gm. soil Plus	100 min.	200 c.c. N. acetic	.00037444	.00146800	.00108366
9	50 gm. soil 0.2 gm	110 min.	200 c.c. N. acetic	.00037004	.00146800	.00109796
10	50 gm. soil $FeSO_4$	70 min.	Water.....	.00036784	.00146800	.00110016

The results indicate that the longer period of shaking with the larger amount of acid tends to remove slightly more ferrous iron from the soil. By shaking for the various lengths of time, the iron is not all removed as indicated by the last three analyses. Since there are a number of steps to this method, there is a greater chance for oxidation than there would be in the first method. Unless the bottles were completely filled with carbon dioxide before shaking there would be an oxidation of the iron to the ferric form. This oxidation cannot be determined because as yet there is no method whereby the ferric iron can be determined in the presence of the ferrous. Since 50 gm. of soil were used in this method there would be some ferric iron present in the soil, but as the soil contained little organic matter, there has probably been no change in this form of iron.

Method IV

In this method a large separatory funnel was used to replace the funnel holding the filter plate in Method I. Thus the acid could be held in contact with the soil for a longer period of time. The apparatus is shown in figure 3.

Fifty gm. of soil and 0.5 gm. of ferrous sulfate were mixed and placed upon the filter, and carbon dioxide allowed to pass through the apparatus from a generator; 200 c.c. of normal acetic acid was allowed to percolate through the soil slowly, about 50-c.c. portions remaining in

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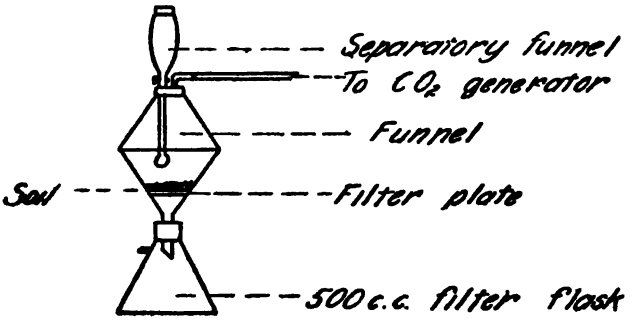

Fig. - 1

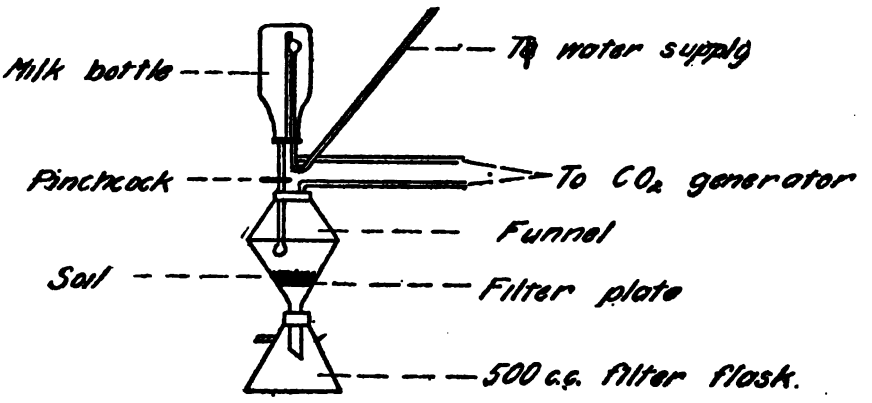

Fig. - 2

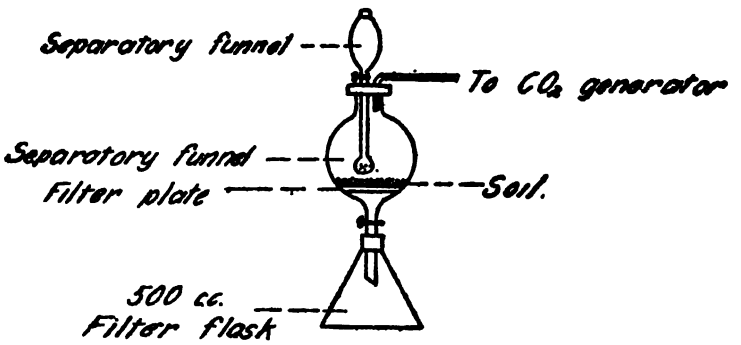

Fig. - 3

Fig. 1.—Diagram of apparatus used in Method I.
Fig. 2.—Diagram of apparatus used in Method II.
Fig. 3.—Diagram of apparatus used in Method IV.

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contact with the soil for 5 minutes each time. Fifty gm. of soil with 0.5 gm. of ferrous sulfate and 10 gm. of peat were treated in the same manner.

TABLE IV
FERROUS IRON CALCULATED ACCORDING TO METHOD IV

.0019822 gm. iron found in filtrate from soil + 0.5 gm. of ferrous sulfate.	
.0014640 gm. iron found in the soil, ferrous sulfate and peat.	
.0005182 gm. decrease due to the addition of peat.	

The organic matter has caused a decrease in the amount of ferrous iron obtained, either through an oxidation or an adsorption of the ferrous sulfate. Similar results were secured by Morris and Doyne (10).

Method V

The total filtrate was used in this method instead of an aliquot as in Method III. Otherwise the two methods are the same. The time of shaking was one hour for each analysis, and the treatment as shown in Table V.

TABLE V
FERROUS IRON CALCULATED ACCORDING TO METHOD V

Treatment	Gm. Fe Found
50 gm. soil + 0.5 gm. $FeSO_4$	.07916445
50 gm. soil + 0.5 gm. $FeSO_4$ + 20 gm. of peat	.03469305
50 gm. soil + 0.5 gm. $FeSO_4$ + 20 gm. of peat + 1 gm. of ferric sulfate	.04634670

In this case there has also been an oxidation due to the organic matter, which is in agreement with results secured by Morris and Doyne, cited in Method IV. From the results obtained in Table V, it is apparent that with soils high in organic matter, the ferrous iron content could not be accurately determined. By adding a ferric compound to the soil with an addition of peat, there is a reduction of the ferric iron. Thus under certain conditions, the ferrous iron obtained upon extraction in the presence of organic matter, may contain some of the ferric iron subsequently reduced.

Method VI

A large glass bottle, 3½ inches in diameter from which the bottom was cut, was fitted into a Büchner funnel as shown in figure 4.

In the funnel was placed a filter paper and a layer of asbestos. One hundred gm. of soil were placed in a shaker bottle of 1000 c.c. capacity and carbon dioxide run in to replace all air; 200 c.c. of normal acetic acid was added and shaken for 10 minutes. The contents of the bottle were made up to the 1000-c.c. mark and connections made as shown. After carbon dioxide had filled the apparatus, the contents of the bottle were drawn over into the funnel and filtered.

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TABLE VI

FERROUS IRON CALCULATED ACCORDING TO METHOD VI

.1839000 gm.	ferrous iron in 0.5 gm. $FeSO_4$.
.0006733 gm.	ferrous iron in soil.
.1845733 gm.	ferrous iron in soil + that in $FeSO_4$.
.0200500 gm.	ferrous iron in filtrate after extraction.
.1645233 gm.	ferrous iron oxidized or absorbed.

The table shows a smaller recovery of the ferrous iron by this method than by any other yet tried. The adsorptive power of the soil has thus far been considered as one of the causes of incomplete recovery of the ferrous iron added. To determine this adsorption, a series was run and results secured as shown in Table VII.

TABLE VII

DETERMINATION OF ADSORPTION OF FERROUS IRON BY SOIL

Sample[1]	Gm. Fe Reclaimed	Minus Fe in Soil	Gm. Fe Held
100 gm. soil	.001057	.....	.....
100 gm. soil + 0.5 gm. $FeSO_4$	.014976	.013919	.169981
100 gm. soil + 1.0 gm. $FeSO_4$	.055064	.054007	.313798
100 gm. soil + 2.0 gm. $FeSO_4$	.165194	.164137	.571463
50 gm. soil + 0.5 gm. $FeSO_4$	.037004	.035947	.147953
50 gm. soil + 1.0 gm. $FeSO_4$	.101299	.100721	.267558
100 gm. soil + 1.0 gm. $FeSO_4$	.036122	.035065	.332735

[1] One hundred fifty c.c. of normal acetic acid was used as a solvent in the first six tests and water in the last one.

From these results it is noted that the adsorptive power of the 100 and 50-gm. samples of soil are in almost direct proportion to the amount of ferrous sulfate added. In the last extraction, where water was used as a solvent, the amount of iron retained by the soil was greater than when 100 c.c. of normal acetic acid was used.

The time of shaking being an important factor, the following experiment was arranged, in which the shaking period varied from 2 to 8 hours, ferrous ammonium sulfate being used, as shown in Table VIII.

TABLE VIII

FERROUS IRON DETERMINATIONS WITH VARIOUS SHAKING PERIODS

Sample	Time Shaken	Average gm. Fe	Gm. Fe Added	Gm. Fe Held by Soil
100 gm. soil + 0.7 gm. $FeSO_4(NH_4)_2SO_4.6H_2O$..	2 hr.	.004350	0.1	.095650
100 gm. soil + 0.7 gm. $FeSO_4(NH_4)_2SO_4.6H_2O$..	4 hr.	.002570	0.1	.097430
100 gm. soil + 0.7 gm. $FeSO_4(NH_4)_2SO_4.6H_2O$..	6 hr.	.002092	0.1	.097908
100 gm. soil + 0.7 gm. $FeSO_4(NH_4)_2SO_4.6H_2O$..	8 hr.	.002026	0.1	.097974
50 gm. soil + 0.7 gm. $FeSO_4(NH_4)_2SO_4.6H_2O$..	6 hr.	.061227	0.1	.038773

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The above results are the averages for two determinations. It is apparent that an increase in the time of shaking increased the oxidation and the extraction is therefore smaller.

Method VII

The 1000-c.c. bottles in the preceeding method were replaced with quart milk bottles. Otherwise these two methods were alike.

The effect due to time of shaking was again determined, using ferrous sulfate.

TABLE IX

FERROUS IRON CALCULATED ACCORDING TO METHOD VII

Sample	Time Shaken	Gm. Fe Recovered	Gm. Fe Held by Soil
100 gm. soil with 600 c.c. N. acetic	2 hr.	.057928	.125972
100 gm. soil with 600 c.c. N. acetic	4 hr.	.052532	.131368
100 gm. soil with 600 c.c. N. acetic	6 hr.	.051650	.132250
100 gm. soil with 600 c.c. N. acetic	8 hr.	.040748	.143152
100 gm. soil with 100 c.c. conc. acetic + 500 c.c. water	4 hr.	.070262	.113638
100 gm. soil with 100 c.c. conc. acetic + 500 c.c. water	7 hr.	.052862	.131038

It is clearly shown that by increasing the time of shaking there is a corresponding increase in the amount of ferrous iron retained. The concentration of the acid apparently has little effect upon the results. The ferric iron in the sample has probably not been reduced since the soil used contained only a small amount of organic matter.

Method VIII

In this method an apparatus was set up as shown in figure 5.

A large glass bottle 18 cm. in diameter, from which the bottom had been cut, was sealed in a Büchner funnel. The method of operation was the same as in Method I. The funnel was provided with an asbestos filter pad and 100 gm. of soil plus 0.5 gm. of ferrous sulfate were placed upon this. Two hundred c.c. of normal acetic acid was used to remove the ferrous iron, which gave the results shown in Table X.

TABLE X

FERROUS IRON CALCULATED ACCORDING TO METHOD VIII

.183900 gm. ferrous iron added in the $FeSO_4$.	
.080394 gm. ferrous iron recovered in the filtrate.	
.103506 gm. ferrous iron not removed.	

This method was repeated five times, and an average taken of the results. The largest amount of ferrous iron that could be obtained by this means of extraction was .080394 gm. To test the various strengths of acids, using this method, a series of determinations were run as shown in Table XI.

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TABLE XI

FERROUS IRON CALCULATED ACCORDING TO METHOD VIII, WITH THE USE OF DIFFERENT ACIDS

Acid Used	Weight of Sample	Gm. Fe Added	Gm. Fe Found	Gm. Fe Not Recovered
N/2 HCl	100 gm.	0.5 gm. $FeSO_4$	.084800	.099100
1 to 3 acetic.....	100 gm.	0.5 gm. $FeSO_4$	.050054	.133846
N/2 acetic	100 gm.	0.5 gm. $FeSO_4$	.047906	.135994

As in the other methods there was 0.13 gm. of ferrous iron that could not be recovered by using acetic acid. The HCl in this case has increased the amount recovered somewhat.

Method IX

Three 100-gm. samples of soil were weighed out and placed in quart milk bottles; 0.1 gm. of ferrous carbonate was added to each one and CO_2 run in to replace the air. Acid was then added and the bottles shaken as shown in Table XII. The contents of the bottles were drawn over into the large Büchner funnel used in Method VIII. The arrangement of the apparatus for this method was the same as in Method VI.

TABLE XII

FERROUS IRON CALCULATED ACCORDING TO METHOD IX

Sample	Fe Added	Time Shaken	Acid Used	Gm. Fe Found	Gm. Fe Not Removed
100 gm. soil	1 gm. $FeCO_3$	6 hr.	N acetic	.0006607	.4825000
100 gm. soil	1 gm. $FeCO_3$	6 hr.	N/2 acetic	.0006607	.4825000
100 gm. soil	1 gm. $FeCO_3$	8 hr.	N acetic	.0007598	.4817402

It is again seen that the strength of acid has little effect on the amount of iron recovered. In the experiment, however, there was a slight increase in the amount of iron extracted by shaking for the longer period of time. This may be due in part to the fact that ferrous carbonate is not readily oxidized by the oxygen of the air.

Method X

Since organic matter is thought to oxidize some of the iron, a method was devised whereby rather insoluble ferrous compounds could be used, and made soluble by heating with acids in an atmosphere of carbon dioxide. The apparatus set up as in figure 6 was used, and 100 c.c. of sand weighed out and placed in the Florence flask. One-tenth gm. of ferrous phosphate was added, the flask placed in position, and carbon dioxide run through the apparatus; 200 c.c. of sulphuric acid was then added through the thistle tube, and boiled for 5 minutes in contact with the sand and iron added. An aliquot of the solution was then drawn

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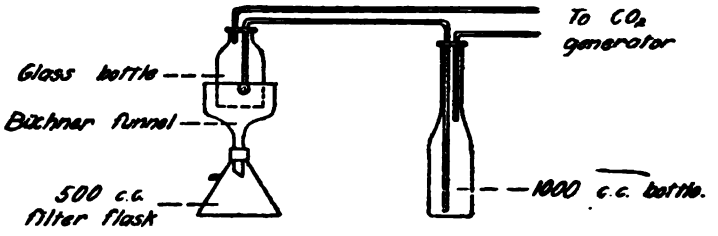

Fig. - 4

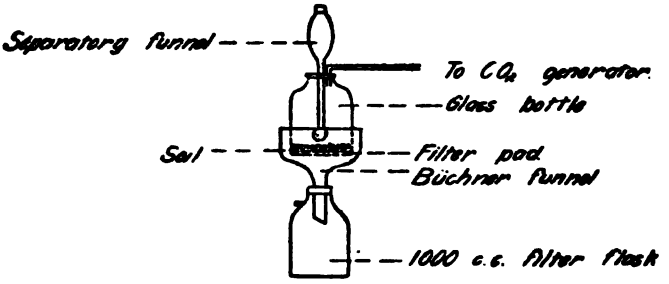

Fig. - 5

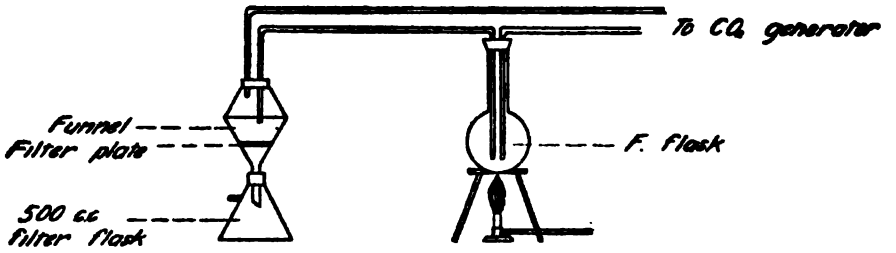

Fig. - 6

Fig. 4.—Diagram of apparatus used in Method VI.
Fig. 5.—Diagram of apparatus used in Method VIII.
Fig. 6.—Diagram of apparatus used in Method X.

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into the funnel. A portion of the filtrate was titrated with potassium permanganate, and the results obtained which appear in Table XIII.

TABLE XIII

FERROUS IRON CALCULATED ACCORDING TO METHOD X

.074974 gm.	Fe found in sand + 0.1 gm. ferrous phosphate.
.025935 gm.	Fe found in sand alone.
.049039 gm.	Fe found in iron compound added.
.046850 gm.	Fe actually in 0.1 gm. ferrous phosphate.
.002189 gm.	Fe error.

These results are the average for three determinations. From Table XIII it is apparent that all the iron added as ferrous phosphate was recovered. There is an error of .002189 gm. and this may be accounted for since the sand varied greatly in its iron content. Upon trying to determine the ferrous iron in the soil by this method, it was found that the sulfuric acid extracted the organic matter, interfering with the permanganate titration.

In summarizing the results from the determinations made, it may be said:

1. Ferrous compounds are adsorbed by the soil, and no determination yet devised will accurately give its ferrous iron content.
2. Organic matter, if present in the soil in any appreciable quantity, will cause an oxidation as well as a reduction of the iron compounds.
3. When extractions are made with acids, the organic matter is carried through and interferes with the permanganate titration.
4. Water is not suitable as a solvent for the ferrous iron, because the iron in this form in the soil probably exists as ferrous carbonate, which is insoluble in water.

Methods for Determining the Ferrifying Power of Soils

As no method was found suitable for extracting the ferrous iron from the soil, whereby the ferrifying power could be determined, attention was turned to the use of water and sand cultures. Ferrous iron, if it exists as such in the soil, is thought to be present in the form of ferrous carbonate. Therefore, this salt will be used as a carrier of iron in this work.

To 100 c.c. of sterile distilled water was added 0.1 gm. of ferrous carbonate and a soil infusion both sterile and fresh. Following incubation the chemical and bacteriological transformations were measured. This was done by filtering the solutions four times whereby the unoxidized ferrous carbonate was removed. The ferric compounds in the filtrate were reduced with stannous chloride to the ferrous form, and titrated with potassium permanganate. After the second filtration calcium car-

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bonate was added, and following the third, sulfuric acid was added and the solution again filtered. In this way a clear filtrate was obtained.

If the amount of iron made soluble by bacterial means is greater than by chemical means, it has been reported as ferrification; if the opposite action, or a reduction, has taken place, it is designated as deferrification.

In the determination where sand is used, 100 gm. of clean quartz sand was sterilized, inoculated with a sterile and fresh infusion, and 0.1 gm. of ferrous carbonate added. The optimum moisture content was made up and maintained throughout the incubation period. The sand was transferred to 1 liter bottles, and distilled water added until the volume reached 400 c.c. The bottles were shaken for one-half hour and the contents filtered. The filtrates were treated in the same manner as those from the series where solutions were used.

The soils used in these series were obtained from the following sources:

Humus plots 102, 107 and 114 are experimental plots, and the soil is a Carrington loam. No. 102 receives 2.8 tons of peat annually, 107 is maintained as a check plot, and 114 receives 4 tons of manure each year. The woodland soil is a Carrington loam, and the samples were taken from a well drained area. The orchard soil is a Miami loam, and is well drained. The corn field soil is a Carrington sandy loam, and has been in rotation for a number of years. Soil from the bank of the creek was chiefly sand with very little organic matter. The pasture soil is of the same type as the cornfield, and has been in permanent pasture for many years.

Ferrification in Soils

Series I

This series was planned to determine the ferrifying power of the soil from humus plot 107, using various kinds of media. One hundred c.c. of distilled water was used in some cases, and in others 100 c.c. of a nutrient solution as indicated in Table XIV. The nutrient solution contained the following salts in the amounts indicated per liter.

K_2HPO_4 0.5 gm.
$MgSO_4$ 0.2 gm.
Na_2CO_3 0.2 gm.
$NaNO_3$ 0.1 gm.
$CaCl_2$ Trace

The incubation period was for 12 days at 27° to 28° C.

This preliminary experiment has shown that $FeCO_3$ would be the most desirable ferrous compound of the two used.

It is apparent from Table XIV that the soil from humus plot 107 has a ferrifying power, and the most definite results are secured when

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distilled water is used as a medium and 0.1 gm. of ferrous carbonate added after sterilization of the water. The nutrient solution did not appear to make the organisms more efficient in transforming the insoluble carbonate to the soluble compound. Where water was used and the ferrous carbonate added before sterilization, there was apparently a change in the carbonate making it more difficult for the organisms to bring about the process of ferrification.

TABLE XIV

FERRIFYING POWER OF SOIL AS DETERMINED WITH DIFFERENT KINDS OF MEDIA

Treatment	Mg. Fe Found	Bacterial Ferrification	% Original Amt. Ferr.
Nutrient solution + $FeCO_3$ sterile	0.275325		
Nutrient solution + $FeCO_3$ + infusion sterile	0.385455		
Nutrient solution + $FeCO_3$ sterile + infusion	1.156365	0.770910	1.5980
Water + $FeCO_3$ sterile	0.220260		
Water + $FeCO_3$ + infusion sterile	0.550650		
Water + $FeCO_3$ sterile + infusion	0.550650	0.000000	0.0000
Nutrient solution + ferrous phosphate sterile	0.275325		
Nutrient solution + ferrous phosphate + infusion sterile	0.440520		
Nutrient solution + ferrous phosphate sterile + infusion	0.715845	0.275325	0.8240
Water + ferrous phosphate sterile	0.330390		
Water + ferrous phosphate + infusion sterile	0.495585		
Water + ferrous phosphate + infusion	0.825975	0.330390	0.9880
Water sterile + ferrous carbonate	0.297351		
Water sterile + infusion sterile + $FeCO_3$	0.495585		
Water sterile + infusion + $FeCO_3$	1.872210	1.376625	2.8530
Water sterile + ferrous phosphate	0.330390		
Water sterile + infusion sterile + phosphate	0.495585		
Water sterile + infusion + ferrous phosphate	1.101300	0.605715	1.8120

Series II

Since the one soil used in the preceding experiment has a ferrifying power, the next question was; do all soils have a ferrifying power, and if so, how do they compare?

Soils were collected from eight different places, representing as far as possible, the extremes in organic matter content, topography, formation, etc., as indicated by the descriptions of these soils on page 562.

The medium for growth of the organisms consisted of 100 c.c. of distilled water to which had been added 0.1 gm. of ferrous carbonate after the water had been sterilized. Incubation was for 12 days, starting December second, at a temperature of 27° to 28° C.

The results from this series are shown in figure 7 together with the organic matter content of the soils. The curves do not show any definite relationship between the organic matter content and the ferrifying power of the soils. It is quite apparent, however, that the different soils have a ferrifying power, which is quite variable. This variation may be the result of many factors. For example, when the samples were taken from humus plots 102 and 107, these plots were almost under water. Thus it

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may be that the organisms in these soils capable of causing an oxidation of iron, were not present in as large numbers as in the better drained soils.

TABLE XV
FERRIFYING POWER OF DIFFERENT SOILS

Source of Soil and Treatment	Av. mg. Iron Found	Ferr. in mg. by Bact.	Deferr. in mg. by Bact.	Per cent of Original Sample	
				Ferr.	Deferr.
Humus plot 102 H_2O + infusion ster. + $FeCO_3$	1.6240				
H_2O ster. + infusion + $FeCO_3$	1.1200	.....	0.5040	.....	1.0440
Humus plot 107 " " "	1.6520				
	0.6720	.....	0.9800	.....	2.0310
Humus plot 114 " " "	1.5680				
	1.7690	0.2010	.....	0.4165	.....
Woodland " " "	1.8480				
	1.4560	.....	0.3920	.....	0.8124
Orchard " " "	1.2320				
	1.4840	0.2520	.....	0.5222	.....
Cornfield " " "	0.5600				
	1.4000	0.8400	.....	1.7409	.....
Bank of creek " " "	1.7360				
	2.2120	0.4760	.....	0.9865	.....
Pasture " " "	0.5040				
	0.9520	0.4480	.....	0.9284	.....

Series III

The incubation period in the preceding series being only one week, it was thought that this may have been one factor limiting ferrification. This

TABLE XVI
FERRIFYING POWER OF DIFFERENT SOILS, DETERMINED WITH AN INCUBATION PERIOD OF 4 WEEKS

Source of Soil and Treatment	Av. mg. Iron Found	Ferr. in mg. by Bact.	Deferr. in mg. by Bact.	Per cent of Original Sample	
				Ferr.	Deferr.
Humus plot 102 H_2O + infusion ster. + $FeCO_3$	1.0920				
H_2O ster. + infusion + $FeCO_3$	0.8400	.....	0.2520	.....	0.5222
Humus plot 107 " " "	0.7000				
	0.9240	0.2240	.....	0.4663	.....
Humus plot 114 " " "	0.7000				
	0.5320	.....	0.1680	.....	0.3482
Woodland " " "	0.4760				
	0.7000	0.2240	.....	0.4663	.....
Orchard " " "	0.5320				
	0.7280	0.1960	.....	0.4062	.....
Cornfield " " "	0.8680				
	1.2320	0.3640	.....	0.7544	.....
Bank of creek " " "	0.8400				
	0.7840	.....	0.0560	.....	0.1160
Pasture " " "	1.0920				
	1.1200	0.0280	.....	0.0580	.....

series was carried out in the same manner as Series II. The incubation period was extended to four weeks, however, being started January 25th. The soils were all frozen when samples were taken for this series.

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As in Series II there is a variation in the ferrifying power of the different soils. This variation is in agreement with Series II in some cases as shown by comparing the curves in figure 7.

With the humus plot 102, there was deferrification in both cases. With plot 107 there was a marked deferrification in Series II and a slight ferrification in Series III. There is no relation between the results from plot 114 or from the woodland soil in either series. The orchard, cornfield, bank of creek and pasture soils all agree very well in their ferrifying power, and the increases and decreases are in approximately the same ratio. The peculiar feature regarding the two curves is that as the incubation period is increased, the ferrifying power of the soil decreases, and more nearly approaches the zero line.

Series IV

According to Fred[1], 2 gm. of potassim acetate per liter of water makes a suitable medium for the growth of iron bacteria. In this series, 100 c.c. of sterile distilled water containing 2 gm. of potassium acetate per liter

TABLE XVII

FERRIFYING POWERS OF DIFFERENT SOILS DETERMINED IN SERIES IV

Source of Soil and Treatment	Av. mg. Iron Found	Ferr. in mg. by Bact.	Deferr. in mg. by Bact.	Per cent of Original Sample	
				Ferr.	Deferr.
Humus plot 102 H_2O + infusion ster. + $FeCO_3$	1.2320				
H_2O ster. + infusion + $FeCO_3$	0.7000	.....	0.5320	.....	1.0922
Humus plot 107 " " "	0.6720				
	1.0360	0.3640	.....	0.7544	.....
Humus plot 114 " " "	1.2320				
	1.1480	.....	0.0840	.....	0.1740
Woodland " " "	0.9800				
	0.8400	.....	0.1400	.....	0.2901
Orchard " " "	0.8120				
	1.0920	0.2800	.....	0.5803	.....
Cornfield " " "	0.8400				
	1.0360	0.1960	.....	0.4062	.....
Bank of creek " " "	0.7560				
	1.1480	0.3920	.....	0.8124	.....
Pasture " " "	0.8680				
	0.8120	.....	0.0560	.....	0.1160

was used as a medium. To this was added 0.1 gm. of $FeCO_3$. The incubation period was for four weeks at 27° and 28° C.

Upon examining the curve for this series in figure 7 it is seen that the soil from humus plots 102, 107 and 114 all give results corresponding to those in the preceeding series. The woodland and orchard soils are in agreement with Series II. In the orchard, cornfield and soil from the bank of the creek, there has been ferrification, though not quite in the

[1] In unpublished data.

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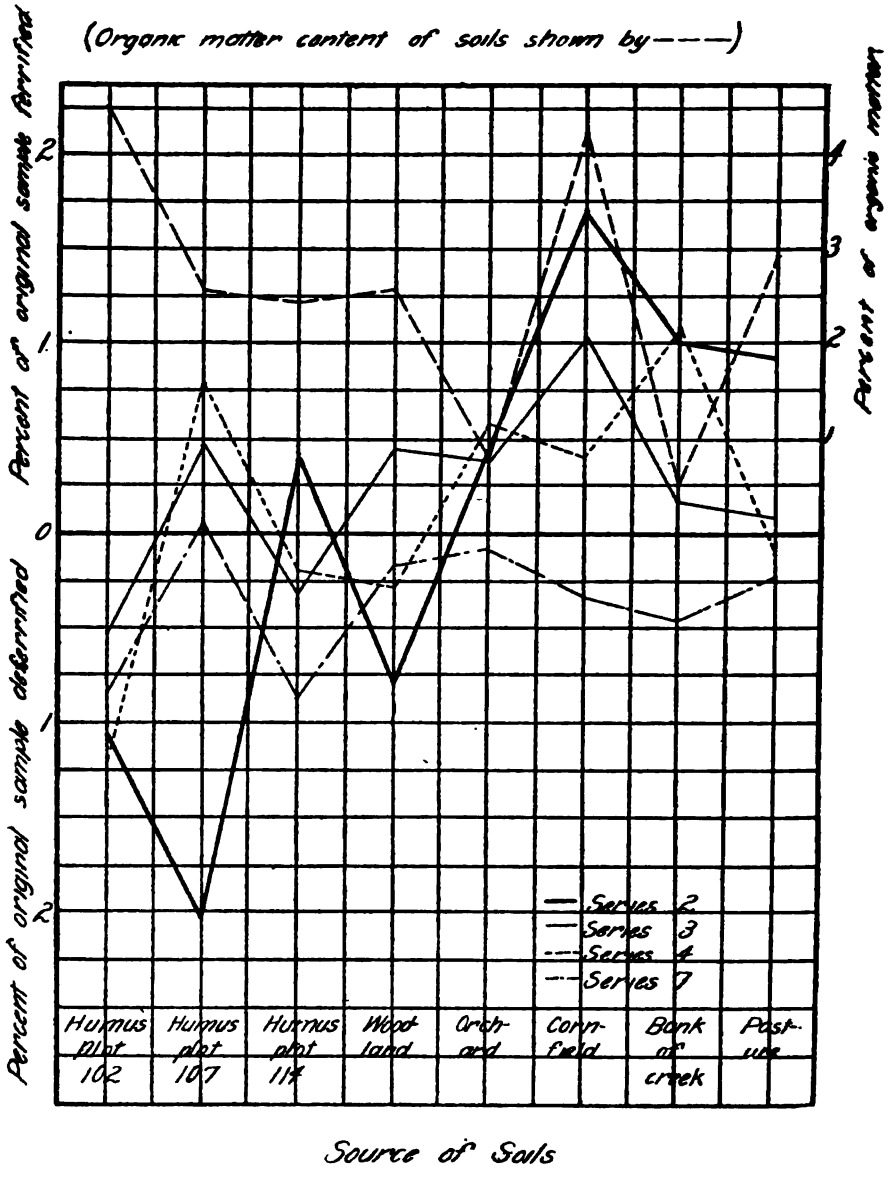

Fig. 7.—Diagram showing ferrification and deferrification in solutions.

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same proportion. From this and the two preceeding series there has been an apparent oxidation of the iron compound, and this change may be influenced by cultivation of the soil.

Series V

In order to bring about ferrification under conditions more nearly comparable to field conditions, clean quartz sand was used in this series as a medium. One-hundred gm. portions were sterilized in tumblers at 15 pounds pressure for 4 hours, 0.1 gm. of ferrous carbonate being added to each tumbler after the sterilization. Sterile and fresh infusions were added to the tumblers as indicated in Table V, and the optimum moisture content made up and maintained throughout the experiment with sterile distilled water. The incubation was for 4 weeks, starting January 25, at a temperature of 27° to 28° C.

TABLE XVIII

FERRIFYING POWER OF DIFFERENT SOILS, DETERMINED WITH SAND AS A MEDIUM

Source of Soil and Treatment	Av. mg. Iron Found	Ferr. in mg. by Bact.	Deferr. in mg. by Bact.	Per cent of Original Sample	
				Ferr.	Defern.
Humus plot 102 Sand + ster. infusion + $FeCO_3$	0.9520				
Sand + fresh infusion + $FeCO_3$	1.4840	0.5320	.....	1.1025	.....
Humus plot 107 " " "	1.3440				
	0.8960	.....	0.4480	.....	0.9280
Humus plot 114 " " "	1.6240				
	1.5120	.....	0.1120	.....	0.2318
Woodland " " "	1.2880				
	1.7360	0.4480	.....	0.9280	.....
Orchard " " "	1.2320				
	1.1200	.....	0.1120	.....	0.2318
Cornfield " " "	1.1760				
	1.6240	0.4480	.....	0.9280	.....
Bank of creek " " "	1.2320				
	1.4840	0.2520	.....	0.5222	.....
Pasture " " "	1.6800				
	1.9600	0.2800	.....	0.5803	.....

In figure 8 the results appear to agree very closely with the organic matter content. That is, where there was a decrease in the amount of organic matter, there was a decrease in ferrification, although not, in the same ratio. As noted in the series where solutions were used, the cornfield soil gave ferrification to the extent of 0.9280 per cent of the original sample.

Series VI

In connection with Series V a similar series was planned with the exception, that in Series VI the optimum moisture content was maintained by using distilled water containing 2 gm. of potassium acetate per liter.

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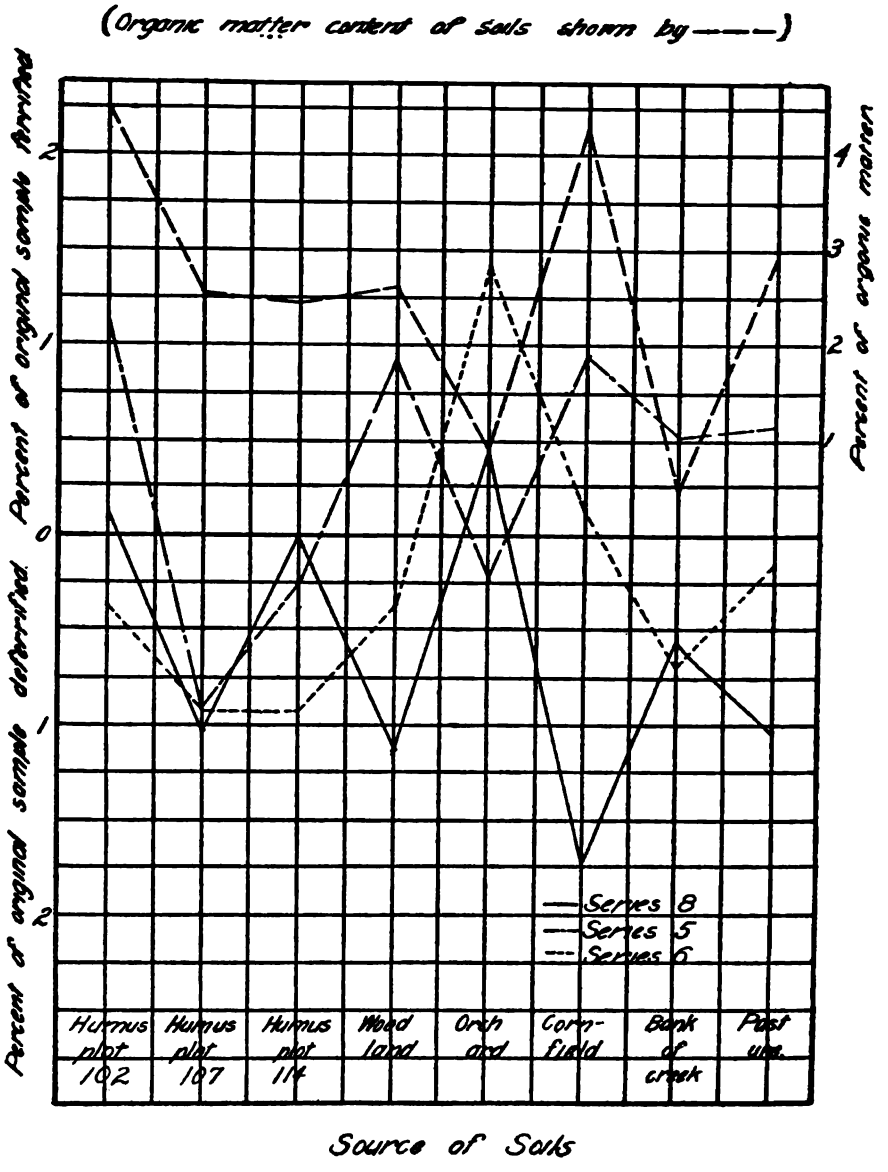

Fig. 8.—Diagram showing ferrification and deferrification in sand.

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The curve for this series in figure 8 cannot be compared with that for Series V except in three cases. With the soil from humus plots 107 and 114 there was deferrification in both cases, in the two series. The cornfield soil again gave slight ferrification, although not as pronounced as in the other series. From these reseults it appears that the potassium acetate has been injurious rather than beneficial to the organisms bringing about ferrification.

TABLE XIX

FERRIFYING POWER OF SOILS AS DETERMINED BY SERIES VI

Source of Soil and Treatment	Av. mg. Iron Found	Ferr. in mg. by Bact.	Deferr. in mg. by Bact.	Per cent of Original Sample	
				Ferr.	Deferr.
Humus plot 102 Sand + ster. infusion + $FeCO_3$	1.2320				
Sand + fresh infusion + $FeCO_3$	1.0640	.....	0.1680	.....	0.3481
Humus plot 107 " " "	1.5680				
	1.1200	.....	0.4480	.....	0.9280
Humus plot 114 " " "	2.5200				
	2.0720	.....	0.4480	.....	0.9280
Woodland " " "	1.4000				
	1.2320	.....	0.1680	.....	0.3481
Orchard " " "	1.2880				
	1.9600	0.6720	.....	1.3927	.....
Cornfield " " "	1.1760				
	1.2320	0.0560	.....	0.1160	.....
Bank of creek " " "	1.7360				
	1.4000	.....	0.3360	.....	0.6963
Pasture " " "	1.7920				
	1.7360	.....	0.0560	.....	0.1160

Series VII

Upon reviewing the work thus far where solutions were used, it appears that the incubation period was too long, since the amount of soluble iron obtained became smaller as the incubation period increased. It may be that the organisms again transfer the soluble iron into an insoluble form. This series was prepared as was Series III, the incubation period in this case being only 7 days instead of 4 weeks.

The results appearing in Table VII and plotted in figure 7 indicate the effect of the short incubation period. It is evident that the bacteria bringing about ferrification have not had an opportunity to develop properly. Consequently, the chemical action has been greater in all cases except one, and in this, it is only slight.

Series VIII

In order to determine the ferrifying power of the soils where sand is used with the short incubation period, a series was prepared similar to Series V, the incubation period being in this case 10 days instead of 4 weeks, as in the Series V.

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The curve for this series in figure 8 clearly shows that the incubation period was not long enough for the most efficient work of the organisms.

TABLE XX

FERRIFYING POWER OF SOILS AS DETERMINED WITH AN INCUBATION OF 7 DAYS

Source of Soil and Treatment	Av. mg. Iron Found	Ferr. in mg. by Bact.	Deferr. in mg. by Bact.	Per cent of Original Sample	
				Ferr.	Deferr.
Humus plot 102 H_2O + infusion ster. + $FeCO_3$	0.8400				
H_2O ster. + infusion + $FeCO_3$	0.4200	.....	0.4200	.....	0.8704
Humus plot 107 " " "	0.8120				
	0.8400	0.0280	.....	0.0580	.....
Humus plot 114 " " "	1.0360				
	0.6160	.....	0.4200	.....	0.8704
Woodland " " "	1.0080				
	0.9240	.....	0.0840	.....	0.1740
Orchard " " "	0.9240				
	0.8960	.....	0.0280	.....	0.0580
Cornfield " " "	0.8400				
	0.6720	.....	0.1680	.....	0.3481
Bank of creek " " "	0.8960				
	0.6720	.....	0.2240	.....	0.4642
Pasture " " "	0.8400				
	0.7280	.....	0.1120	.....	0.2321

This is most noticeable in the cornfield soil. While ferrification was quite marked in Series V with this soil, it shows a decided deferrifying power with the shorter incubation period.

TABLE XXI

FERRIFYING POWER OF SOILS AS DETERMINED WITH AN INCUBATION PERIOD OF 10 DAYS

Source of Soil and Treatment	Av. mg. Iron Found	Ferr. in mg. by Bact.	Deferr. in mg. by Bact.	Per cent of Original Sample	
				Ferr.	Deferr.
Humus plot 102 Sand + ster. infusion + $FeCO_3$	1.5680				
Sand + fresh infusion + $FeCO_3$	1.6240	0.0560	.....	0.1160	.....
Humus plot 107 " " "	2.1840				
	1.6800	.....	0.5040	.....	1.0445
Humus plot 114 " " "	1.7920				
	1.7920	0.0000	.....	0.0000	.....
Woodland " " "	2.1280				
	1.5680	.....	0.5600	.....	1.1606
Orchard " " "	1.6800				
	1.9040	0.2240	.....	0.4642	.....
Cornfield " " "	2.5200				
	1.6800	.....	0.8400	.....	1.7409
Bank of creek " " "	2.0160				
	1.7362	.....	0.2798	.....	0.5798
Pasture " " "	2.2400				
	1.7360	.....	0.5040	.....	1.0445

Comparing the curves for all the solution tests in figure 7 for the various soils, it is seen that in general the results agree quite well. Just

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why there should be deferrification with the soil from humus plot 107 in Series II cannot be explained.

When the results for the sand tests in figure 8 are examined there seems to be no definite relationship between them. Series V is the only one which can be considered, since the short incubation period and the effects produced by the potassium acetate eliminate the other two.

Series IX

To determine the ferrifying power of bacteria in solutions where pure cultures are used, 100 c.c. of distilled water was sterilized in flasks and

TABLE XXII

FERRIFYING POWER OF BACTERIA

Name of Organism	Mg. Iron Found	Minus Blank	Per cent of Original Sample Ferrified
B. communior	0.980	0.196	0.4062
B. coli	1.315	0.531	1.1005
Cladothrix dichotoma..	1.315	0.531	1.1005
B. subtilis	1.036	0.252	0.5222
Proteus vulgaris	1.148	0.364	0.7544
B. cloaceae	1.176	0.392	0.8331
B. pyocyaneus	1.315	0.531	1.1005
B. phosphorescence	1.232	0.448	0.9287
Check	0.784	.....	.....

0.1 gm. of $FeCO_3$ added. The bacteria were brought into suspension, 100 c.c. of sterile water being used, and 1 c.c. of this was added to each flask in duplicate.

The results show that *Bacillus coli, Bacillus pyocyaneus* and *Cladothrix dichotoma* are the most efficient in bringing about ferrification.

Series X

In many of the changes going on in the soil, the molds may take a very important part. To determine the ferrifying power of molds, four

TABLE XXIII

FERRIFYING POWER OF MOLDS

No.[1] Mold	Treatment	Av. mg. Iron Found	Ferr. in mg. by Mold	Per cent of Original Sample Ferr.
1	H_2O ster. + 0.1 gm. $FeCO_3$ + 0.2 gm. dext.....	1.5400	.....	.....
2	H_2O ster. + 0.1 gm. $FeCO_3$ + 0.2 gm. dext. + 1 c.c. culture	5.7680	4.2280	8.7626
3	H_2O ster. + 0.1 gm. $FeCO_3$ + 0.2 gm. dext. + 1 c.c. culture	3.1920	1.6520	3.4238
4	H_2O ster. + 0.1 gm. $FeCO_3$ + 0.2 gm. dext. + 1 c.c. culture	7.6720	6.1320	12.7088
5	H_2O ster. + 0.1 gm. $FeCO_3$ + 0.2 gm. dext. + 1 c.c. culture	2.2120	0.6720	1.3927

[1] The molds used in the above experiment were:
1. Check. 2. Chaetomium. 3. Trichoderma. 4. Aspergillus niger. 5. Rhizopus.
Two-tenths gm. of dextrose was added to each flask to furnish organic matter for the molds.

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representative ones were chosen, and a series planned and results secured as shown in Table XXIII.

The results suggest that the molds may be an important factor in bringing about the transformation of the rather insoluble ferrous compounds to the soluble forms, in the soil.

Further work may show that they are as efficient as the bacteria, if not more so, in bringing about ferrification.

Conclusions

1. Ferrification and deferrification are processes common to the soils used in this study. The degree of ferrification and deferrification may depend upon many factors, such as organic matter content of the soil, cultivation, moisture, temperature and food supply.

2. An incubation period of one week in solutions is not sufficient time to allow the organisms to develop and bring about ferrification.

3. There is no relation between the organic matter content of the soil, and its ferrifying power, when solutions are used.

4. The cultivated soil has the greatest ferrifying power.

5. Sterile water to which is added 0.1 gm. of ferrous carbonate is the best medium for the growth of the bacteria causing ferrification in solutions.

6. Sand, with the addition of 0.1 gm. of ferrous carbonate and maintained at an optimum moisture content, with distilled water, represents more nearly the conditions of moisture, temperature and aeration in the field, than do the solutions.

7. When sand is used as a medium for the growth of the bacteria, it appears that the ferrifying power of the soil is related to its organic matter content. Where there was a high percentage of organic matter, there was a corresponding increase in ferrification. When the soil was low in organic matter, deferrification took place.

8. The use of pure cultures of bacteria indicate that the common soil organisms, as well as the iron bacteria, are able to bring about ferrification.

9. Molds as well as bacteria are capable of oxidizing ferrous iron.

Literature Cited

(1) Benecke, W.
1895. Die zur Ernährung der Schimmelpilze nothwendigen Metalle. *In* Jahrb. Wiss. Bot. [Pringsheim], Bd. 28, p. 487-530.

(2) Ellis, D.
1907. A contribution to our knowledge of thread bacteria, I. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 19, p. 502-518.

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(3) ELLIS, D.
1910. A contribution to our knowledge of thread bacteria, II. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 26, p. 321-329.
(4) GRIFFITH, A. B.
1889. A Treatise on Manures. 389 p., 15 fig., London, New York.
(5) HALL, A. D.
1909. Fertilizers and Manures. 384 p., 9 fig., New York.
(6) HALLIGAN, J. E.
1912. Soil Fertility and Fertilizers. 397 p., 23 fig. Easton.
(7) LIPMAN, J. G.
1911. Suggestions concerning the terminology of soil bacteria. *In* Bot. Gaz., v. 51, p. 454-460.
(8) MOLISCH, H.
1892. Die Pflanze in ihrer Beziehungen zum Eisen. 119 p. Jena.
(9) MOLISCH, H.
1910. Die Eisenbakterien. 83 p. Jena.
(10) MORRISON, C. G. T., and DOYNE, H. C.
1914. Ferrous iron in soils. *In* Jour. Agr. Sci., v. 6, p. 97-101.
(11) RAULIN, J.
1869. Etudes chimiques sur la vegetation. *In* Ann. Sci. Nat. Bot., s. 5, t. 11, p. 93-299.
(12) RULLMANN, W.
1912. Ueber Eisenbakterien. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 33, p. 277-289.
(13) RUSSELL, E. J., and HUTCHINSON, H. B.
1909. The effect of partial sterilization of soil on the production of plant food. *In* Jour. Agr. Sci., v. 3, p 111-114.
(14) SCHORLER, B.
1904. Beiträge zur Kenntnis der Eisenbakterien. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 12, p. 681-695.
(15) SCHREINER, O., and FAILYER,
1905. Colorimetric, turbidity and titration methods used in soil investigations. U. S. Dept. Agr. Bur. Soils Bul. 31, 60 p., 1 pl., 5 fig.
(16) WILEY, H. W.
1916. Principles and Practice of Agricultural Analysis: Vol. I, Soils. 636 p. 92 fig. Easton.

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A VEGETATION EXPERIMENT ON THE AVAILABILITY OF NITROGENOUS FERTILIZERS IN AN ARID SOIL[1]

By

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In a series of papers which either have recently appeared (2, 3) or are soon to appear (4, 5) the senior author alone, or together with P. S. Burgess, has furnished experimental evidence which amounted in general to the following:

1. The nitrification method, in the direct soil culture, for determining the relative availabilities of nitrogenous fertilizers of the organic or ammonia type was entirely feasible, and more logical and specific in nature than other methods employed heretofore.

2. By the use of that method an important distinction is observable between humid and arid soils as regards ability to nitrify the nitrogen of soils.

3. Humid soils, contrary to the general impression created by Hilgard's teachings, are superior in nitrifying power to the arid soils when they are considered by and large.

4. Dried blood and other high grade nitrogenous fertilizers nitrify relatively slowly in arid soils and rapidly in humid soils.

5. The situation is reversed when the low grade organic nitrogenous fertilizers or sulfate of ammonia are employed. These nitrify much more readily in arid than in humid soils.

6. The foregoing results and conclusions appeared to justify the recommendation to farmers that, on the truly arid soils of California and under circumstances in which availability was the desideratum, low grade organic nitrogenous fertilizers or sulfate of ammonia be employed.

7. It was also pointed out that nitrates, and especially nitrate of soda, did not under most circumstances in arid soils, behave like the other nitrogenous fertilizers mentioned in the foregoing paragraph. The reason for this is that nitrates, being immediately soluble in the soil moisture when applied to the soil, begin to rise with the moisture upwards from the bottoms of irrigation furrows and tend to accumulate in the dry surface soil. The latter cannot, of course, be of use to plants since it is used largely as a dust mulch, and hence the surface soil may contain an ample quantity of nitrates and still the roots of plants in the subsoil may starve for want of available nitrogen. That this accumulation of

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nitrates occurs in the surface layers of arid soils has been well known through the publications of Hilgard on alkali salts in soil. It has, however, been more fully explained and emphasized recently by the investigations of McBeth at the Citrus Experiment Station, Riverside, Cal.

8. Sulfate of ammonia which showed itself to be unusually well suited to nitrification in arid soils according to our results, was applied on the senior author's recommendation to orchards in which "little leaf" or in which "chlorosis" was seriously injuring the trees. In nearly all such cases, the trees were brought back to a normal condition by the soil treatment. In some of these cases high grade organic nitrogenous fertilizers, nitrates, and barnyard manure had been used for years with little or no effect.

9. In general the senior author has become convinced through our various studies that, owing to the low nitrogen content of arid soils, not enough available nitrogen is elaborated and not enough remains in the feeding zone of the roots, also, that in many cases in which the soil's total nitrogen content is high, our climatic conditions and tillage have operated to lower the nitrifying power of the soils to such a degree that insufficient available nitrogen is maintained in the root zone for normal crop production.

The method employed in the various nitrification studies upon which the foregoing conclusions were based is as follows: 100-gm. portions of the soil to be studied are either incubated under optimum moisture conditions for one month at 28° to 30° C. and the nitrates formed determined, or the soil portion as above is mixed with 1 per cent of the organic fertilizer to be tested and otherwise similarly treated. Usually soils both with and without fertilizer are studied in the case of every type.

The validity of this method has been called in question by Kelley (*1*) on the ground that it allows an excessive use of fertilizer as compared with soil material, and hence cannot be used as a criterion for field studies in which relatively small quantities of fertilizer per acre are employed. Our reply to this argument is that undoubtedly the conditions of our experiments are unlike those of the field, and that the use of small quantities of fertilizers in the soils to be studied will soon gain in favor and probably be adopted for nitrification work. However, we also claim that our results are valid for determining sufficiently accurately the *relative* availabilities of different organic nitrogenous fertilizers; and particularly as a reliable guide to the choice of nitrogenous fertilizers for soils.

In order, however, to test this idea more definitely, we decided to carry out a vegetation experiment with an arid soil which gave us the following results by our method of determining the nitrifiability of nitrogen fertilizers. The soil contained .03 per cent of total nitrogen as determined by our modification of the Gunning method.

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It is clear from these data that cottonseed meal and steamed bone meal are practically equally nitrifiable by our method and that sulfate of ammonia, although used in smaller amounts, yields about as much nitrate as the other two. Dried blood, however, encourages a loss of even the small quantity of nitrate originally present in the soil. If, therefore, our method could be employed as a guide in the choice of nitrogenous fertilizers for the soil here studied, it would indicate that steamed bone meal, cottonseed meal and sulfate of ammonia are of about equal value, and that dried blood is decidedly inferior. It is of course obvious that the method is not perfect because of the fact that when used in small quantities dried blood gives better crop results, as Table II shows, than the soil without fertilizer, yet the latter soil gives better nitrification results than the blood by our method. On the other hand, it must be observed that in a special test with some of the same fertilizers in the

TABLE I

NITRIFICATION OF NITROGEN IN DIFFERENT FERTILIZERS IN OAKLEY BLOW SAND

Fertilizer Used	Amount Per cent	Nitrate N. Found Mg.	Nitrate N. Produced Mg.
None	...	0.42	0.36
None	...	0.60	0.55
Dried Blood	1.0	....	....
Dried Blood	1.0	Trace	....
Steamed Bone Meal	1.0	3.00	2.95
Steamed Bone Meal	1.0	2.40	2.35
Cottonseed Meal	1.0	2.24	2.19
Cottonseed Meal	1.0	2.40	2.35
$(NH_4)_2SO_4$	0.1	2.24	2.19
$(NH_4)_2SO_4$	0.1	2.48	2.43

same soil, in all cases small quantities being used equivalent to the same amount of nitrogen which is found in .05 gm. of dried blood, the cottonseed meal is distinctly superior to the others, dried blood takes second place, and the sulfate of ammonia is distinctly last.

Let us now see how these results harmonize, if at all, with the vegetation tests. The latter were carried out in 9-inch earthenware pots which were paraffined to prevent absorption of moisture and salts by the walls of the pot. About ten pounds of the Oakley blow sand were placed in each pot and every treatment was run in triplicate. A selected strain of the Beldi variety of barley was employed as the crop plant. The fertilizers were applied in different amounts in two series. In one series amounts equivalent to those employed in the laboratory tests were incorporated with the soil. In the second series amounts of the different fertilizers were used which contained a quantity of nitrogen equivalent to that used when dried blood is applied at the rate of 800 pounds per acre. The fertilizers tested were dried blood, steamed bone meal, cottonseed meal,

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sulfate of ammonia, nitrate of soda, and nitrate of calcium. From a number of seeds planted at first the plants which appeared were thinned to four per pot.

It almost goes without saying that scarcely any growth, and in some cases none, was obtained in the series containing the large fertilizer applications. In those pots in which plants came up normally they grew to a height of three or four inches and then gradually turned yellow and withered. This occurred primarily in the pots containing the organic fertilizers and was doubtless due to the absorption of excessive amounts of ammonia by the plants, which exercised a marked toxic effect. That this was probably so is indicated by the strong odor of ammonia which was always noticeable in the vicinity of the pots under consideration. The possibility also exists, of course, that organic compounds of a toxic nature formed from the decomposition of the fertilizers may have been the cause of the poisoning of the plants. In the case of the inorganic fertilizers the effects were probably nothing more nor less than alkali effects which are already well known.

In the second series of pots the plants came up and developed normally but the difference between the different treatments was visible almost from the very start. Until the plants arrived at a height of eight or ten inches, the steamed bone meal treatment was slightly but distinctly superior to the other ones employed, but later in the growth of the plants the sulfate of ammonia and cottonseed meal treatments caught up with and finally surpassed the first named. The dried blood treatment showed itself from the start to be inferior to the three treatments just given. The nitrate of soda treatment was about equal to that of the dried blood in efficiency, but the calcium nitrate was inferior to both. Table II gives the results of studies on the grain, straw and root yields obtained.

Considering first the total weights of dry matter produced, we note the striking effect which nitrogenous fertilizers exercise on barley growth in the Oakley sand. That available nitrogen is sorely needed in that soil there can be no question. In the second place, we note clearly the superiority of sulfate of ammonia and cottonseed meal to all the other fertilizers. The two fertilizers just named are but very slightly different from each other. Thirdly, it should be noted that steamed bone meal comes next in order to the first two fertilizers named. Unfortunately, the plants in one of the three pots in the steamed bone meal treatment were injured and the yield made of no value to a closer appraisal of the actual effects of the steamed bone meal nitrogen. The dried blood treatment was distinctly behind the three just discussed as regards the total yield of dry matter. The nitrate of soda treatment was about equal to the dried blood treatment with respect to the yield of total dry matter, and the calcium nitrate treatment was distinctly inferior to both. We

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see, therefore, in the first place a close resemblance between total dry matter yields and the appearances of the plants in the pots. In the second place we note a very close resemblance between the availability of the nitrogen in the different fertilizers as shown by both the laboratory and the vegetation methods.

On the basis of the grain and straw yields alone, and disregarding the root yields, sulfate of ammonia, cottonseed meal and steamed bone meal are even closer together in regard to availability than they are when the

TABLE II

SHOWING AVAILABILITY OF DIFFERENT NITROGENOUS MATERIALS FOR BARLEY IN OAKLEY SAND

No. and Treatment	Weight of Grain and Straw	Average Weight of Grain and Straw	Weight of Grain	Average Weight of Grain	Weight of Roots	Average Weight of Roots	Total Dry Weight Produced	Average Total Dry Weight
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1. Blood	22.6	22.7	6.59	6.63	4.00	3.87	26.60	26.57
2. Blood	25.5	....	6.19	....	4.80	....	30.30	.....
3. Blood	20.0	....	7.12	....	2.80	....	22.80	.....
4. Steamed Bone Meal	24.6	26.6	6.24	7.59	3.90	3.70	28.50	30.85
5. Steamed Bone Meal	Lost	....	....	....	Lost	....	Lost	.....
6. Steamed Bone Meal	28.6	....	8.93	....	4.60	....	33.20	.....
7. Cottonseed Meal	31.0	29.9	8.44	7.61	6.70	6.13	37.70	36.30
8. Cottonseed Meal	30.0	....	8.63	....	6.70	....	36.70	.....
9. Cottonseed Meal	28.6	....	5.75	....	5.00	....	33.60	.....
10. $(NH_4)_2SO_4$	30.0	31.2	9.17	8.62	8.40	7.27	38.40	38.80
11. $(NH_4)_2SO_4$	32.1	....	8.45	....	7.00	....	39.10	.....
12. $(NH_4)_2SO_4$	31.5	....	8.27	....	7.40	....	38.90	.....
13. $NaNO_3$	24.0	22.3	5.93	6.19	4.50	4.50	28.50	26.76
14. $NaNO_3$	19.8	....	6.70	....	4.10	....	23.90	.....
15. $NaNO_3$	23.0	....	5.96	....	4.90	....	27.90	.....
16. $Ca(NO_3)_2$	22.3	18.5	8.34	6.52	5.00	4.00	27.30	22.80
17. $Ca(NO_3)_2$	17.6	....	6.73	....	4.50	....	22.10	.....
18. $Ca(NO_3)_2$	15.5	....	4.49	....	3.50	....	19.00	.....
19. Control	12.5	12.1	4.00	3.63	1.20	1.10	13.70	13.20
20. Control	11.7	....	3.25	....	1.00	....	12.70	.....

weights of total dry matter are employed as criteria. Nevertheless, we find again that sulfate of ammonia is superior to all the other fertilizers, cottonseed meal is a close second, and steamed bone meal a good third in order of availability. Dried blood and nitrate of soda are again equal as between themselves, and considerably behind the others. The calcium nitrate treatment gives distinctly the poorest of all yields with the exception, of course, of the untreated soil.

As regards the production of grain alone, the situation is almost the same as in the case of the combined grain and straw production. Sulfate of ammonia stands first and cottonseed meal and steamed bone meal, which are about equal to each other, stand as close seconds. In the

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case of the other fertilizers we find that dried blood and nitrate of soda are again about equal, and that calcium nitrate is not inferior to them as it was in the case of the combined grain and straw yields, but is about equal to them.

In the case of the root yields we find the first evidence of strong differentiation between sulfate of ammonia and cottonseed meal on the one hand and steamed bone meal on the other. Sulfate of ammonia appears to exert its most marked effects with respect to availability on the root yields. Cottonseed meal is a fairly close second, and in about the same class, but the other fertilizers are distinctly in the third class and about the same among themselves.

In general, it would appear therefore that as regards their availability for barley production on the Oakley sand, the fertilizers here under examination (the nitrates being excluded for obvious reasons) stand nearly in the same relation to each other in the vegetation tests as they do in the laboratory tests as carried out by our method. We deem it justifiable, therefore, to urge the use of the nitrification method in some form for the determination of the relative availabilities of organic and inorganic nitrogenous fertilizers. Our results would lead us also to ask if we have not by means of them at least brought forward some evidence in favor of employing the nitrification method as a criterion in studies such as those considered in this paper?

The Relative Availabilities of the Different Fertilizers as Determined by Our Experiments

Adopting now the method of Voorhees in his book on fertilizers and also that employed by other agricultural chemists in the past, we may determine the relative values of the different fertilizers used by us for

TABLE III

RELATIVE AVAILABILITIES OF NITROGEN FERTILIZERS IN OAKLEY SAND
(Values based on dry yields of straw and grain)

Nitrate of Sodium	100
Nitrate of Calcium	82+
Sulfate of Ammonia	139+
Dried Blood	101+
Cottonseed Meal	134+
Steamed Bone Meal	119+

the Oakley soil, barley being the crop plant. Assuming the value of nitrate of soda to be 100, as is usually done, the values for the other fertilizers would be those shown in Table III.

The figures in Table III speak for themselves and appear to us to bear out the contention which is supported also by our laboratory data that the low grade organic nitrogenous fertilizers and sulfate of ammonia

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are far more available in arid soils like the Oakley blow sand than the high grade organic nitrogenous fertilizers. They also show that mere solubility in water does not under arid soil conditions necessarily constitute the highest availability. This seems to be supported by the data given for sodium and calcium nitrates.

SUMMARY

1. Vegetation experiments with barley on the availability of nitrogenous fertilizers in the Oakley blow sand (an arid soil) show the low grade organic nitrogenous fertilizers and sulfate of ammonia to be far more available than dried blood.

2. This confirms the values as to *relative availabilities* of these fertilizers obtained by our laboratory nitrification method.

3. Nitrates, and especially nitrate of calcium, are far less available in the Oakley soil than are the other fertilizers with the exception of dried blood, which is about equal to nitrate of soda but superior to nitrate of calcium.

4. These data furnish further evidence in support of our proposal to adopt the nitrification method in some form for the determination of the relative availabilities of fertilizer and soil nitrogen and for determining the needs of a soil for available nitrogen.

LITERATURE CITED

(1) KELLEY, W. P.
1916. Some suggestions on methods for the study of nitrification. *In* Science, n. s., v. 43, p. 30-33.

(2) LIPMAN, C. B.
1915. The nitrogen problem in arid soils. *In* Proc. Nat. Acad. Sci., v. 1, no. 10, p. 477-480.

(3) LIPMAN, C. B., and BURGESS, P. S.
1915. The determination of availability of nitrogenous fertilizers in various California soil types by their nitrifiability. Cal. Agr. Exp. Sta. Bul. 260, p. 107-127.

(4) LIPMAN, C. B., and BURGESS, P. S.
1916. Ammonifiability versus nitrifiability as a test for the relative availability of nitrogenous fertilizers. *In* Soil Sci., (soon to appear).

(5) LIPMAN, C. B., BURGESS, P. S., and KLEIN, M. A.
1916. Comparison of the nitrifying powers of some humid and some arid soils. *In* Jour. Agr. Research, v. 7, no. 2, p. 47-82.

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THE INFLUENCE OF VARIOUS CATIONS UPON THE RATE OF ABSORPTION OF AMMONIUM ION BY SOIL[1]

By

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The mechanism of absorption has recently been carefully studied. Way's (8, 9) purely chemical hypothesis held the field for many years, and while his "double silicates" were never actually shown to exist in the soil, it was assumed that the reactive substances of similar constitution known as zeolites, and found naturally in volcanic districts, were normal soil constituents and responsible for the absorption. Some justification for this view was obtained when Hall and Gimingham (4) found that the interaction between ammonium sulphate and clay followed the ordinary law of mass action. But the old experiments of Weinhold (10) and more recent ones of Cameron and Patten (3) showed that this law is not obeyed over a wider range of concentration, and another hypothesis has therefore been developed.

Van Bemmelen (7) has demonstrated a close parallelism between the various interchanges and absorptions shown by the soil and those shown by colloids; and there is considerable evidence in other directions that some of the soil constituents, especially the clay, possess all the properties of colloids. Now the time relation of rate of the absorption by colloids is generally expressed by the equation

$$x = Kt^m$$

where x is the amount absorbed, t is the time, K and m are constants which depend upon the concentration and kind of colloid and solution used.

The relation $x = Kt^m$ was found first by Cameron and Bell (1, 2) and later confirmed by Ostwald (5) from the investigations of Goppelsroeder, to hold for the relation between the amount of fluid absorbed by a column of sand or of a strip of filter-paper and the time during which the fluid remained in contact with a portion of its surface. Robertson (6) has also found that this relation for the rate of solution of casein by sodium hydroxide is primarily determined by the rate at which the casein particles absorb the solvent.

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If we grant that absorption by a soil is mainly a colloidal phenomenon, it is of interest to study the influence of various cations upon the rate of absorption of the ammonium ion by the soil, since cations have a great influence upon the properties of colloidal substances. It is also important and interesting from the standpoint of agricultural practice to know the characteristic effects of one cation upon the absorption of another, since it is very seldom that single nutrient salts are used as fertilizer. Accordingly, the following experiments were undertaken.

The soil employed was Hagerstown loam. The solution to be absorbed was 0.1N. ammonium chloride solution. The salts employed were chlorides of sodium, potassium, magnesium, calcium and aluminium in the concentrations of 0.01N, 0.05N, and 0.1N. Fifty gm. of the soil were placed in 300-c.c. Erlenmeyer flasks: to these were added 100 c.c. of the solutions to be examined. The flasks were shaken frequently at room temperature and at stated intervals the supernatant fluid was filtered and analyzed according to the ordinary distillation method, using magnesium oxide and distilling the ammonia into standard acid. The results obtained are shown in Table I.

In the column headed "calculated" are given the value of x calculated from the above formula, the constants K and m being determined from the results of the all observations, employing for this purpose the form

$$\log x = \log K + m \log t.$$

It will be seen from the results given that the observed and calculated values of x agree very closely. It is, therefore, evident that the relation between the time during which the fluid has remained in contact with the soil and the amount of ammonia absorbed is expressed by $x = Kt^m$ when the chloride of another base is present with the ammonium chloride solution. In such an instance, however, the presence of the salt caused retardation of the rate of absorption of ammonia, and the greater the concentration of the salt, the greater was the retardation.

In regard to their power to cause retardation of the rate of absorption, there is a difference in the cations used. The retarding effect of the cations employed increases in the following order:

$$\mathrm{Na} < \mathrm{Mg} < \mathrm{Ca} < \mathrm{Al} < \mathrm{K},$$

instead of the usual order in colloid chemistry:

$$\mathrm{Na} < \mathrm{K} < \mathrm{Mg} < \mathrm{Ca} < \mathrm{Al},$$

potassium, in the former case, being the strongest, whereas in the latter it is the weakest of all except sodium.

The magnitudes of the constants K and m are affected by the salt. In the case of all salts tested except potassium chloride, the values of K and m decrease as the concentration of salt increases. In the case of potassium chloride the value of K decreases as the concentration of salt increases, while that of m increases.

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TABLE I

AMMONIA ABSORBED: AMOUNT FOUND COMPARED WITH AMOUNT CALCULATED

Mg. of Ammonia Absorbed by 50 gm. of Soil from 100 c.c. of Solution

1. Solution: 0.1 N. NH_4Cl $K = 1.622$ $m = 0.4046$			9. Solution: 0.1 N. NH_4Cl + 0.05 N. $MgCl_2$ $K = 1.660$ $m = 0.2874$		
Time, Hours	Found	Calculated	Time, Hours	Found	Calculated
24	5.95	5.97	24	4.15	4.19
48	8.08	7.91	48	5.20	5.12
72	9.35	9.53	72	5.80	5.83
2. Solution: 0.1 N. NH_4Cl + 0.01 N. NaCl $K = 1.698$ $m = 0.3763$			10. Solution: 0.1 N. NH_4Cl + 0.1 N. $MgCl_2$ $K = 1.216$ $m = 0.2393$		
24	5.78	5.72	24	2.65	2.63
48	7.68	7.41	48	3.10	3.11
72	8.50	8.81	72	3.45	3.43
3. Solution: 0.1 N. NH_4Cl + 0.05 N. NaCl $K = 1.318$ $m = 0.3736$			11. Solution: 0.1 N. NH_4Cl + 0.01 N. $CaCl_2$ $K = 1.738$ $m = 0.3571$		
24	4.42	4.40	24	5.53	5.50
48	5.95	5.69	48	7.14	7.03
72	6.38	6.74	72	8.08	8.27
4. Solution: 0.1 N NH_4Cl + 0.1 N. NaCl $K = 1.072$ $m = 0.3589$			12. Solution: 0.1 N. NH_4Cl + 0.05 N. $CaCl_2$ $K = 1.514$ $m = 0.3062$		
24	3.40	3.41	24	4.08	4.06
48	4.40	4.37	48	5.19	5.00
72	5.10	5.15	72	5.53	5.78
5. Solution: 0.1 N. NH_4Cl + 0.01 N. KCl $K = 2.399$ $m = 0.235$			13. Solution: 0.1 N. NH_4Cl + 0.1 N. $CaCl_2$ $K = 1.122$ $m = 0.2521$		
24	5.10	5.12	24	2.55	2.53
48	5.95	6.01	48	2.98	3.01
72	6.80	6.71	72	3.40	3.38
6. Solution: 0.1 N. NH_4Cl + 0.05 N. KCl $K = 0.7943$ $m = 0.364$			14. Solution: 0.1 N. NH_4Cl + 0.01 N. $AlCl_3$ $K = 1.799$ $m = 0.3316$		
24	2.55	2.57	24	5.27	5.24
48	3.40	3.30	48	6.60	6.59
72	3.83	3.91	72	7.60	7.67
7. Solution: 0.1 N. NH_4Cl + 0.1 N. KCl $K = 0.05623$ $m = 0.629$			15. Solution: 0.1 N. NH_4Cl + 0.05 N. $AlCl_3$ $K = 1.084$ $m = 0.3211$		
24	0.43	0.43	24	3.00	3.06
48	0.68	0.68	48	3.90	3.81
72	0.85	0.85	72	4.40	4.42
8. Solution: 0.1 N. NH_4Cl + 0.01 N. $MgCl_2$ $K = 1.778$ $m = 0.3541$			16. Solution: 0.1 N. NH_4Cl + 0.1 N. $AlCl_3$ $K = 0.3508$ $m = 0.435$		
24	5.60	5.59	24	1.40	1.43
48	7.15	7.11	48	2.00	1.93
72	8.25	8.36	72	2.33	2.36

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Differentiating the equation

$$x = Kt^m$$

we find

$$\frac{dx}{dt} = Kmt^{m-1}$$

in other words, the product Km, which we may term the coefficient of absorption in this case expresses the constant proportionality between the velocity of absorption and the exponent of the time during which the soil has been contacted with the fluid.

TABLE II

COEFFICIENT OF ABSORPTION: OBSERVED VALUES COMPARED WITH CALCULATED VALUES

Solution	K	m	$K \times m$	
			Found	Calculated
1. NaCl $K_1m_1 = 0.656$ $\alpha = 2.05$ $\beta = 19.21$				
0.1 N. NH_4Cl	1.622	0.4046	0.656	0.656
0.1 N. NH_4Cl + 0.01 N. NaCl	1.698	0.3763	0.639	0.635
0.1 N. NH_4Cl + 0.05 N. NaCl	1.318	0.3736	0.492	0.505
0.1 N. NH_4Cl + 0.10 N. NaCl	1.072	0.3589	0.265	0.259
2. KCl $K_1m_1 = 0.656$ $\alpha = 9.234$ $\beta = -30.75$				
0.1 N. NH_4Cl	1.6220	0.4046	0.656	0.656
0.1 N. NH_4Cl + 0.01 N. KCl	2.3990	0.2350	0.564	0.567
0.1 N. NH_4Cl + 0.05 N. KCl	0.7943	0.3640	0.289	0.271
0.1 N. NH_4Cl + 0.10 N. KCl	0.0562	0.6290	0.035	0.040
3. $MgCl_2$ $K_1m_1 = 0.656$ $\alpha = 2.878$ $\beta = 8.452$				
0.1 N. NH_4Cl	1.622	0.4046	0.656	0.656
0.1 N. NH_4Cl + 0.01 N. $MgCl_2$	1.778	0.3541	0.630	0.626
0.1 N. NH_4Cl + 0.05 N. $MgCl_2$	1.660	0.2874	0.477	0.491
0.1 N. NH_4Cl + 0.10 N. $MgCl_2$	1.216	0.2393	0.291	0.284
4. $CaCl_2$ $K_1m_1 = 0.656$ $\alpha = 3.700$ $\beta = 0.635$				
0.1 N. NH_4Cl	1.622	0.4046	0.656	0.656
0.1 N. NH_4Cl + 0.01 N. $CaCl_2$	1.738	0.3571	0.621	0.619
0.1 N. NH_4Cl + 0.05 N. $CaCl_2$	1.514	0.3062	0.464	0.469
0.1 N. NH_4Cl + 0.10 N. $CaCl_2$	1.122	0.2521	0.283	0.280
5. $AlCl_3$ $K_1m_1 = 0.656$ $\alpha = 6.562$ $\beta = -14.28$				
0.1 N. NH_4Cl	1.622	0.4046	0.656	0.656
0.1 N. NH_4Cl + 0.01 N. $AlCl_3$	1.799	0.3316	0.597	0.592
0.1 N. NH_4Cl + 0.05 N. $AlCl_3$	1.084	0.3211	0.348	0.364
0.1 N. NH_4Cl + 0.10 N. $AlCl_3$	0.351	0.4350	0.153	0.143

Since the value of K decreases more rapidly than m increases, even in the case of potassium chloride, with increasing concentration of salt, the effect of all the salts is to cause a decrease in the value of the coefficient of absorption, a decrease which is a definite function of the concentration of salt employed.

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The relationship between the value of the coefficient of absorption and the concentration of salt added to the fluid may be defined by the interpolation equation.

$$K_1m_1 - Km = \alpha c + \beta c^2$$

where K_1m_1 is the coefficient of absorption for the standard solution (in this case 0.1N. NH_4Cl), Km. is its value when a given salt has been added in concentration c, α and β are constants dependent upon the nature of the salt added.

In Table II the observed values of the coefficient of absorption are compared with the values calculated from the equation; the values of the constants α and β being calculated by the method of least squares from all of the observations obtained with the given salt.

TABLE III

VALUES OF CONSTANTS

	K_1m_1	α	β
Na	0.656	2.050	19.210
Mg	0.656	2.878	8.452
Ca	0.656	3.700	0.635
Al	0.656	6.562	—14.280
K	0.656	9.234	—30.750

The observed and calculated values of Km are very close. These results reveal another striking difference between the effects of various cations upon the rate of absorption of ammonia by the soil, for whereas NaCl, $MgCl_2$ and $CaCl_2$ in increasing concentration decrease the rate of absorption with a positive acceleration, $AlCl_3$ and KCl decrease the rate of absorption with a negative acceleration.

SUMMARY

I. The relation between the time which a solution of ammonium chloride has remained in contact with soil and the amount of ammonia absorbed by the soil is expressed by the equation $x = Kt^m$ even in the presence of another chloride in solution.

2. The presence of other chlorides decreases the rate of absorption of ammonia from ammonium chloride solution by the soil. The retardation increases with the concentration of salt employed.

3. Salts differ in their power of causing retardation of the rate of absorption of ammonia. The retarding effect of the salts employed increases in the following order:

$$Na < Mg < Ca < Al < K.$$

4. The value of $K \times m$ which we term the coefficient of absorption in this case, decreases with increasing concentrations of the salts, the acceleration of the decrease being positive in the cases of NaCl, $MgCl_2$ and $CaCl_2$, and negative in the cases of $AlCl_3$ and KCl.

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In conclusion, the author wishes to express his thanks to Dr. Edmund C. Shorey for the kindness which he has shown during this experiment.

Literature Cited

(1) Bell, J. M., and Cameron, F. K.
1906. The flow of liquids through capillary spaces. *In* Jour. Phys. Chem., v. 10, p. 658-674.

(2) Cameron, F. K., and Bell, J. M.
1905. The mineral constituents of the soil solution. U. S. Dept. Agr. Bur. Soils Bul. 30, 70 p.

(3) Cameron, F. K., and Patten, H. E.
1907. The distribution of solute between water and soil. *In* Jour. Phys. Chem., v. 11, no. 8, p. 581-593, 4 fig.

(4) Hall, A. D., and Gimingham, C. T.
1907. The interaction of ammonium salts and the constituents of the soil. *In* Jour. Chem. Soc. [London], v. 91-92, no. 534, p. 677-687.

(5) Ostwald, W.
1908. Ueber das Zeitgesetz des kapillaren Aufstiegs von Flüssigkeiten und über die Beziehungen desselben zur chemischen Konstitution der Letzteren nach Versuchen von F. Goppelsroeder. *In* Ztschr. Chem. Indus. Kolloide, Jahrg. 2, Sup. 2, p. 20-39.

(6) Robertson, T. B.
1910. The rate of solution of casein. *In* Jour. Phys. Chem., v. 14, no. 5, p. 377-392, 2 fig.

(7) Van Bemmelen, J. M.
1910. Die Absorption: Gesammelte Abhandlungen über Kolloide und Absorption von J. M. von Bemmelen . . . mit Unterstützung des Verfassers neu Herausgegeben von dr. W. Ostwald. Dresden.

(8) Way, J. T.
1850. On the power of soils to absorb manure. *In* Jour. Roy. Agr. Soc., v. 11, p. 313-379.

(9) Way, J. T.
1852. On the power of soils to absorb manure. *In* Jour. Roy. Agr. Soc., v. 13, p. 123-143.

(10) Weinhold, A.
1863. Einwirkung von Ammoniaksalzen auf Boden. *In* Landw. Vers. Stat., Bd. 5, p. 308-317.

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PERCY EDGAR BROWN, PH.D.

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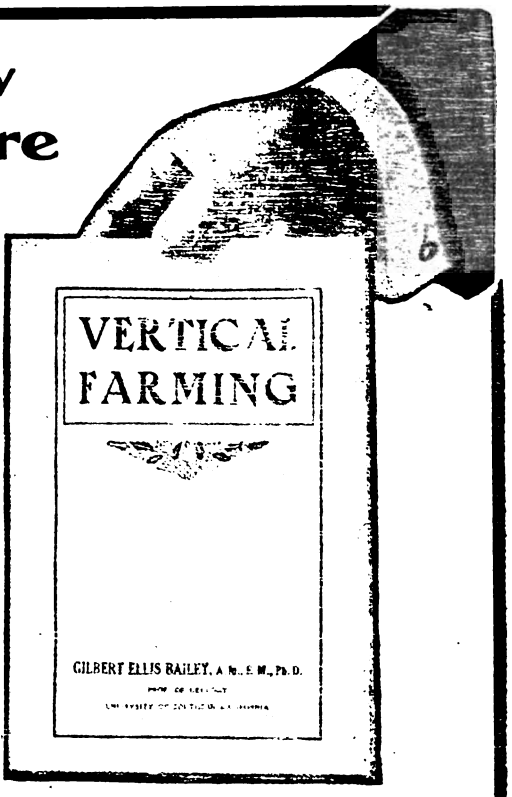

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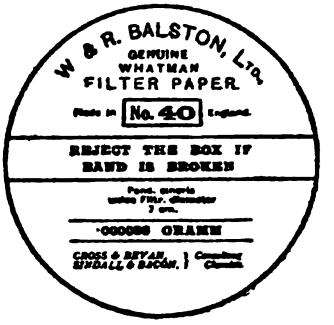

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